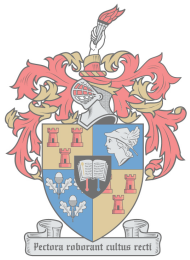
The impact of UV-light on grapevine berry and wine metabolites

by

Chandré Honeth



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Declaration

By submitting this dissertation, I declare that the entirety of the work contained therein is my own, original work, with the exception of the contributions made by others as stated below. The study was conceived by Professor Melane Vivier who along with Dr. Philip Young contributed critical evaluation of the results and research throughout the study. Dr Zelmari Coetzee along with Dr Philip Young helped with the initial application of vineyard treatments, climatic logger installation and berry sampling. Ms Varsha Premsagar assisted in the processing of the berry samples and also the sample preparation for the major sugar, organic acid and photosynthetic pigment analysis. Ms Anke Berry helped in berry sampling, the sample preparation for the berry volatile organic compounds and amino acids as well as in the winemaking process. Dr Hans Eyeghe-Bickong assisted in the UPLC, HPLC and GC-MS analysis as well as in the integration of volatile organic compounds, phenolics and amino acid data. Dr Anscha Zietsman provided the method for the berry cell wall CoMPP analysis and also conducted the monosaccharide analysis of the berry cell wall tissues. Dr Lucky Mokwena from the Central Analytical Facility helped develop a GS-MS method for the analysis of volatile aroma compounds in the juice matrices. Dr Martin Kidd performed the repeated measures ANOVA on the different datasets. The berry polyphenolic compounds were analysed at the Oxidative Stress Research Centre at the Cape Peninsula University of Technology. Juice glutathione was analysed at the LC-MS laboratory at the Central Analytical Facility by Dr Marietjie Stander. The wine volatile compound analysis was conducted by Ms Lynzey Isaacs and Mr Hugh Jumat. The wine sensory evaluation was supervised and the data generated by Ms Jeanne Brand from the Department of Viticulture and Oenology Sensory Laboratory.

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Summary

Within their natural environments, plants are constantly challenged by a multitude of stress factors and have therefore evolved different adaptive strategies to mitigate potential damage as well as to optimise resource utilisation. Sunlight, being one of these abiotic factors, is fundamental to plant functioning, however also represents a potential source of stress and damage. Understanding light stress and consequent plant responses have therefore received considerable attention. The impacts of light on plant development have been studied comprehensively in model systems as well as crop plants. As one of the most commercially important fruit crops, grapevine has received considerable attention and significant headway has been made in recent years to profile the potential responses of grapevine tissues to light quantity and quality, specifically UVB. Despite this progress, scope for further exploration into the impacts of varying light quality exposure on berry growth and composition, as well as the extended effects into the wine matrices still exists. The purpose of this study was therefore to examine the impacts of a modulated exposure level (quantity of light) in combination with modulated quality of light (UVB presence or attenuation) on Sauvignon Blanc grape berry growth and metabolite composition during the development and ripening processes, as well throughout the wine-processing steps, ending with a sensorial description of the wines. The distinctive varietal style of Sauvignon Blanc has been well characterised in relation to light exposure, making this cultivar an ideal study system for evaluating the impacts of UVB radiation.

The trial was designed using a field-omics approach where an experimental system in a cool-climate Sauvignon Blanc vineyard was previously validated to study berry metabolism under high and low light exposure in the bunch zones. This provided an advantageous base from which to evaluate the grape berry responses to UVB radiation under these two light regimes by strategically installing UVB-attenuating acrylic sheets over the bunch zone, thereby creating the following four distinctive bunch microclimates, namely high light and low light microclimates, which served as the controls for the high and low light -UVB microclimates, respectively. Meso- and microclimatic monitoring confirmed that the intended light conditions were indeed achieved in the various microclimates. When evaluating the high light and low light environments separately, the data confirmed the successful attenuation of UVB in each condition while light exposure remained unaffected by the UVB attenuating sheets.

The metabolic responses of the berries under the different microclimates were evaluated by profiling and quantifying primary and secondary metabolites in the whole berries during the developmental and ripening period by sampling at the green, véraison and ripe berry stages over three consecutive seasons. Major sugars and organic acids, photosynthetic pigments, volatile organic compounds, amino acids and polyphenolics were profiled and quantified in the samples and subjected to statistical and multivariate data analyses to reveal developmentally responsive metabolites, and/or metabolites that responded to

the variable light quantity and quality exposure level. It was clear that in addition to developmental patterns, variations in exposure and UVB levels lead to particular changes in berry metabolite compositions.

The results extended the current understanding of UVB responses in grapevine berries by showing that during the green developmental stage, certain carotenoids implicated in photoprotection responded to the variation in light exposure and that the UVB signal specifically was implicated in the photoinhibition response linked to the violaxanthin cycle. Interestingly, under lower light conditions, a similar UVB dependency was seen for the accumulation of lutein epoxide, a xanthophyll linked to acclimation in shade conditions. The primary metabolites as well as the chlorophylls and major carotenoids were mostly unaffected by UVB radiation, indicating that the berries successfully acclimated to their different microclimates. The metabolic profiles of the photoprotective compounds however suggested that the berries in the UVB attenuation microclimates were possibly more prone to stress, particularly in the low light UVB attenuated environment.

The ripe berries also responded to UVB attenuation, but in a different way to the green berries. These responses were furthermore influenced by the level of light exposure. In the ripe berries, the formation of compounds known to have antioxidant and/or “sunscreening” properties were negatively impacted when UVB was attenuated. This was most notable in the high light environment where ambient UVB levels lead to an increase in polyphenolics as well as in certain berry volatile compounds including monoterpenes and norisoprenoids. Similarly, the amino acid composition of the ripe berries was differentially modulated by UVB, specifically regarding the branched chain amino acids and GABA, which may be implicated in the mitigation of stress through their roles as metabolites or signalling compounds. Overall, the results indicated a switch in berry employed acclimation strategies to UVB between the early and late stages of development. The primary objective of the green berries appeared to be the maintenance of photosynthetic activity, whereas the ripe berries shifted their metabolism to accumulate compounds involved in blocking UVB and maintaining the antioxidant status of the tissues as protective measures. The skins and pulp tissues of the ripe berries were also subjected to cell wall profiling techniques, but no indication of altered cell wall monomer or polymer profiles were detected for the different microclimates.

The ripe grapes from the four microclimates were used to conduct a grape to juice to wine metabolite profiling analyses, using a typical Sauvignon Blanc vinification work-flow and including a sensory description of the resultant wines. Juice samples were generated at three pre-fermentation processing steps and evaluated for amino acids, polyphenolics, volatile compounds and glutathione. The results firstly confirmed that the four microclimates yielded four unique juice matrices. Secondly, by tracking the metabolites through the three juice possessing steps, evidence of the inherent dynamic nature of the

juice matrices was revealed, implying the presence of chemical or biological processes which influence susceptible compounds during processing. Additionally, the variations in both light quantity and quality altered the juice environment by possibly changing the juice oxidation status and the juice lipidome, which also impacted the outcome of certain compounds in the wine. The chemical analysis of the wine focused on the fermentation derived compounds and the results confirmed the significant influence of the microclimate on the chemical compositions of the wines. The most notable impacts were noted in the young wines where a higher content of esters was seen with ambient UVB exposure in both the high light and low light microclimates. These results could potentially be related to amino acid composition of the juices, however significant changes occurred in the finished wines during aging.

Sensorial analysis of the final bottled wines following aging revealed perceptible differences associated with the four different microclimates. The results reiterated the characteristic aromatic changes which occur in Sauvignon Blanc wines in relation to the variability of light quantity, but also highlighted the significant impact of specifically UVB on wine sensorial characteristics. In the high light microclimates, ambient UVB exposure was strongly associated with tropical aromatic wines, while the attenuation of UVB generated wine with certain similarities to those of the low light microclimate. This indicated that the UVB component of light was necessary for the formation of compounds responsible for the tropical aromas. Furthermore, the low light microclimate wines were generally described as more green in character, however the attenuation of UVB significantly intensified these aromas. Overall, the results show the significant influence of berry microclimate on grape berry composition, leading to altered juice and wine matrices and ultimately perceivable differences in the wines.

The findings of this study therefore provided new insights into the underlying metabolic mechanisms employed by grape berries to acclimate to UVB radiation, revealing the employment of phenotypic plasticity by Sauvignon Blanc. The results furthermore highlighted the influence of UVB on juice and wine compositional properties and also provided novel insights into the grape-juice-wine transitions of certain metabolites in Sauvignon Blanc.

Opsomming

Plante word voortdurend binne hulle natuurlike omgewing uitgedaag deur 'n verskeidenheid stresfaktore en het dus verskillende strategieë ter aanpassing ontwikkel om potensiële skade te verminder en ook om die gebruik van hulpbronne te optimaliseer. Sonlig, ‘n abiotiese faktor, is fundamenteel belangrik vir plantfunksionering, maar kan ook 'n potensiële bron van stres en skade wees as dit oormatig voorkom. Dit lei daartoe dat die onderwerp van ligstres en die gevolglike reaksie van die plant, aansienlike aandag geniet. Die impak van lig op plantontwikkeling is reeds omvattend in modelsisteme sowel as gewasplante bestudeer. Omdat druiwe een van die kommersieel belangrikste vrugte gewasse is, het dit aansienlike aandag ontvang en is daar in onlangse jare groot vooruitgang gemaak om die potensiële reaksie van wingerdweefsels op ligkwaliteit en kwantiteit aan te dui, ook spesifiek op UVB bestraling. Ten spyte van hierdie vordering bestaan daar nog steeds ‘n behoefte om meer te weet van die impak van blootstelling aan verskillende ligfrekwensies op korrelgroei en samestelling, asook die gevolglike impakte op die wynmatrikse. Die doel van hierdie studie was dus om die impak van 'n gemoduleerde vlak van beligting (lig kwantiteit), in kombinasie met ‘n gemoduleerde lig kwaliteit (gemodifiseerde frekwensiespektrum ten opsigte van UVB-teenwoordigheid) op die Sauvignon Blanc druiwekorrel se groei en metabolietsamestelling te ondersoek. Die analises het gefokus op die ontwikkelings- en rypwordingsprosesse, asook gedurende die wyn voorbereidingsstappe en die uiteindelike sensoriese beskrywing van die wyne. Die kenmerkende kultivar-gekoppelde styl van Sauvignon Blanc, wat beïnvloed word deur blootstelling aan lig, is goed gekarakteriseer en maak dus hierdie kultivar 'n ideale kandidaat om te bestudeer vir die evaluering van die impak van UVB-bestraling.

'n Veld-omika (“Field-omics”) -benadering is gebruik om die studie te ontwerp en deur te voer op ‘n eksperimentele perseel van ‘n koel-klimaat Sauvignon Blanc-wingerd wat reeds voorheen gevalideer is om korrelmetabolisme, onder hoë en lae ligblootstelling, in die trossone te bestudeer. Hierdie gevalideerde perseel is gepas bevind vir die evaluering van die druifkorrels se reaksies op UVB-bestraling onder die hoë en lae ligkondisies. Deur die strategiese installering van akriel UVB-uitsluitingspanele oor die trossones, is vier kenmerkende tros mikroklimate geskep, naamlik ‘n hoë lig en ‘n lae lig mikroklimaat, wat dan ook onderskeidelik die kontroles was vir die hoë en lae lig-sonder-UVB mikroklimate. Meso- en mikroklimaat monitering het bevestig dat die beplande ligtoestande inderdaad in die verskillende mikroklimate bereik is. Die evaluering van die hoë- en lae lig-sonder UVB omgewings het bevestig dat UVB bestraling feitlik afwesig in die trossones agter die akrielpanele was, terwyl die res van die ligfrekwensies nie deur die UVB uitsluitings panele beïnvloed is nie.

Die metaboliese reaksies van die korrels op die verskillende mikroklimate is geëvalueer deur die primêre- en sekondêre metabolietprofiele van die heel korrels te kwantifiseer gedurende die

ontwikkelings- en rypwordingstydperk. Monsters is geneem tydens die groen, véraison- en ryp stadia, oor drie opeenvolgende seisoene. Die primêre suikers en organiese sure, fotosintetiese pigmente, vlugtige organiese verbindings, aminosure en polifenoliese komponente is geïdentifiseer en gekwantifiseer in die monsters en statistiese en multiveranderlike data-analise is gedoen om ontwikkelings-responsiewe metaboliete en/of metaboliete te identifiseer wat op die veranderlike ligkwantiteit en -kwaliteit gereageer het. Dit was duidelik dat verskille in ligkwantiteit en UVB vlakke tot veranderings in ontwikkelingspatrone gelei het, asook tot spesifieke veranderinge in die metabolietsamestelling van die druifkorrels.

Die resultate lei tot ‘n beter begrip van die reaksie van druiwekorrels op UVB bestraling deur te wys dat sekere karotenoïede wat by fotobeskerming betrokke is op die variasie in ligblootstelling reageer tydens die groen ontwikkelingsfase en dat die UVB-sein spesifiek met die foto-inhibisie-reaksie, gekoppel aan die vioolaksien-siklus, geassosieer is. Interessant genoeg, onder laer ligstoestande, is 'n soortgelyke UVB-afhanklikheid gesien vir die opeenhoping van luteïenepoksied, 'n xantofil betrokke by aanpassings by skaduwee toestande. Die primêre metaboliete, asook die chlorofille en primêre karotenoïede was meestal nie deur UVB-bestraling beïnvloed nie, wat aandui dat die korrels suksesvol ge-akklimatiseer het tot hulle verskillende mikroklimate. Die metaboliese profiele van die fotobeskermingsverbindings het egter aangedui dat die korrels in die mikroklimate waar UVB uitgesluit was moontlik meer geneig was tot stres, veral in die lae lig sonder-UVB omgewing.

Die ryp korrels het op ‘n ander manier as die groen korrels op UVB-uitsluiting gereageer en die reaksie was ook beïnvloed deur die vlak van ligblootstelling. In die ryp korrels is die vorming van verbindings wat optree as antioksidante en/of "sonskerm" eienskappe het, negatief beïnvloed wanneer UVB uitgesluit was. Dit was veral opvallend in die hoë-lig omgewing waar die ongemoduleerde UVB-vlakke gelei het tot hoër vlakke van polifenoliese verbindings en volataliele organiese komponente soos monoterpene en norisoprenoïede in die korrel. Net so was die aminosuursamestelling van die ryp korrels differensieel gemoduleer deur UVB, spesifiek ten opsigte van die vertakte-ketting aminosure en GABA, wat by stresverligting betrokke kan wees deur middel van hul rolle as metaboliete of seinverbindings. Algeheel dui die resultate daarop dat die druifkorrel verskillende UVB akklimasie strategieë aanwend tussen die vroeë en laat stadiums van ontwikkeling. Die primêre doelwit van die groen korrels blyk die instandhouding van fotosintetiese aktiwiteit te wees, terwyl die ryp korrels hul metabolisme verskuif deur beskermende verbindings te produseer wat UVB kan uitblok en ook die antioksidant status van die weefsel kan onderhou as beskermingsmaatreëls. Die selwandprofiele van die dop- en pulpweefsels van die ryp korrels is ook bepaal, maar daar was geen aanduiding van ‘n verandering in selwandmonomeer of -polimeerprofiele in reaksie tot die verskillende mikroklimate nie.

Die ryp druiwe van die vier mikroklimate is ook gebruik om 'n druif-tot-sap-tot-wyn metabolietprofiel op te stel, deur gebruik te maak van 'n tipiese Sauvignon Blanc-wynbereidingsplan en 'n sensoriese beskrywing van die finale wyne is ook gedoen. Sap monsters is by drie voor-fermentasie verwerking stappe ontleed vir aminosure, glutatioon, polifenoliese- en vlugtige verbindings. Die resultate het eerstens bevestig dat die vier mikroklimate vier unieke sapmatrikse opgelewer het. Tweedens, deur die metaboliete te volg deur die drie sapvoorbereidings-stappe, is die inherente dinamiese aard van die sapmatrikse geopenbaar, en dit impliseer die teenwoordigheid van chemiese of biologiese prosesse wat sensitiewe verbindings tydens die sapverwerking beïnvloed. Daarbenewens het die variasies in beide ligkwaliteit en kwantiteit die sapsamestellings verander deur moontlik die sap-oksidasie status en die sap lipidoom te verander, wat ook weer ‘n uitwerking op sekere verbindings in die wyn het. Die chemiese analise van die wyn het gefokus op die verbindings wat tipies gedurende gisfermentasie geproduseer word en die resultate het die beduidende invloed van die mikroklimaat op die chemiese samestellings van die wyne, bevestig. Die mees noemenswaardige impak was die hoër konsentrasie van esters in die jong wyne van beide die hoë lig en lae lig mikroklimate onderworpe aan ongemodifiseerde UVB blootstelling. Hierdie resultate kan potensieel verband hou met die aminosuursamestelling van die sappe, maar dit was ook duidelik dat tydens die veroudering van die wyne betekenisvolle veranderinge plaasvind oor tyd.

Sensoriese analise van die finale, gebotteleerde wyne na veroudering het opmerklike verskille wat geassosieer kon word met die vier verskillende mikroklimate, geopenbaar. Die resultate bevestig die kenmerkende aromatiese veranderinge wat in Sauvignon Blanc-wyne voorkom as gevolg van die modulering van ligkwantiteit, maar het ook die aandag gevestig op die beduidende impak van spesifiek UVB op wynsensoriese eienskappe. In die hoë ligmikroklimate met ongemodifiseerde UVB-blootstelling was daar ‘n sterk assosiasie met tropiese aromatiese wyne, terwyl die wyne wat gemaak is van druiwe waar UVB geblok was, sekere ooreenkomste gehad het met dié van die lae-lig-mikroklimaat. Dit het aangedui dat die UVB-komponent van lig noodsaaklik is vir die vorming van verbindings wat verantwoordelik is vir die tropiese aroma. Verder is die lae-lig mikroklimaatwyne algemeen beskryf as meer groen in karakter, maar die uitsluiting van UVB het hierdie aromas aansienlik versterk. In die algemeen, wys die resultate die beduidende invloed van die korrel-mikroklimaat op die samestelling van die druiwekorrel, wat lei tot veranderde sap- en wynmatrikse en uiteindelik tot waarneembare verskille in die wyne.

Die bevindings van hierdie studie het gelei tot nuwe insigte in die onderliggende metaboliese meganismes wat in druiwekorrels gebruik word om aan te pas by UVB-bestraling en dit het veral die fenotipiese plastisiteit van Sauvignon Blanc onderstreep. Die resultate het ook die invloed van UVB op die samestelling van sap en wyn beklemtoon en nuwe insig gegee in hoe sekere Sauvignon Blanc metaboliete “vloei” vof omskakel vanaf die druif tot die sap tot die uiteindelike wyn.

"True education is a kind of never ending story
— a matter of continual beginnings,
of habitual fresh starts, of persistent newness."
— J.R.R. Tolkien

This dissertation is dedicated firstly to my parents for their endless love, support and encouragement.

Secondly, to my husband Mark for his enduring patience, encouragement and love.

Biographical Sketch

Chandré Honeth was born in Kimberley, South Africa and matriculated from Kimberley Girls' High School in 2006. Chandré received a BSc degree in Agriculture from the University of Stellenbosch in 2010, after which she enrolled for an MSc in Viticulture which was obtained in 2012. On completion of this degree, she continued her studies by enrolling for a PhD in Viticulture.

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Preface

This dissertation is presented as a compilation of 7 chapters. Each chapter is introduced separately and is written according to the style of the journal Frontiers in Plant Science. Chapter 3 was published in Frontiers in Plant Science.

Chapter 1	**General introduction and project aims**
Chapter 2	**Literature review** Light stress effects on grapevine organs and metabolism
Chapter 3	**Research results** Field-grown grapevine berries use carotenoids and the associated xanthophyll cycles to acclimate to UV exposure differentially in high and low light (shade) conditions
Chapter 4	**Research results** UVB attenuation impacts on berry amino acids and cell wall composition
Chapter 5	**Research results** A comparison of Sauvignon Blanc juice composition, analysed at three juice-processing steps to evaluate the impacts of UVB attenuation in high and low light microclimates
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Chapter 1

General introduction and project aims

1.1 Introduction

Within their natural environments, plants are exposed simultaneously to a multitude of stress conditions which can be both abiotic (temperature, drought, light) and biotic (pathogenic attack). The inherent sessile nature of plants has required that they evolve different strategies to deal with these external conditions and also optimise resource utilization to remain productive and thriving. The acclimation of plants to abiotic and biotic stress has therefore received much attention, with many studies focusing on various crop plants. Significant efforts have been invested in improving plant performance to ensure optimal crop yield and thereby meet global demands (Ort et al., 2015). A ubiquitous theme in recent years has been the effect of climate change on crop productivity and a number of studies have concentrated on increases in temperature, water scarcity, higher CO_2 levels and impacts of changes in light exposure (Bornman et al., 2015; Parmesan and Hanley, 2015; Zandalinas et al., 2017).

Light quantity and quality represents one of the most dynamic abiotic factors capable of influencing plant functioning, physiology, behavior and development and is generically linked to "exposure". Foremost is light's involvement in photosynthesis whereby plants are able to harvest light energy and convert it into chemical energy to be utilized for various activities. Light however serves not only as a source of energy, but also provides information to the plant through sensing and signaling processes (Hernando et al., 2017; Kami et al., 201f0). This consequently allows plants to perceive their light environment and respond accordingly to maintain optimal photosynthesis and mitigate potential damage. These light-mediated responses and mechanisms employed are independent from photosynthesis and collectively fall under the term "photomorphogenesis."

Light is made of different spectral components and light sensing in plants is made possible by the presence of several photoreceptors which detect specific wavelengths of light ranging from the near-UVB (280–315 nm) to far-red (FR) (~750 nm). Recent studies have started delving into the influences of these individual spectral components and new information regarding the impacts thereof have started emerging, changing the archetypal way in which experiments are designed, methods are developed and results are interpreted (Hideg, Jansen & Strid, 2013 and references therein).

Plant responses to ultraviolet radiation (UVR) have received particular attention due to concerns of higher levels reaching earth as a result of climate change and the depletion of stratospheric ozone (Caldwell et al., 1989; Jansen et al., 1998; Jordan, 2002). Approximately 6% of solar radiation reaching earth is within the UV spectrum. This can be sub-divided into three different groups, namely ultraviolet

A (UVA), ultraviolet B (UVB) and ultraviolet C (UVC), each of which is absorbed in the atmosphere to varying degrees (Moan, 2001). Having a shorter wavelength, UVB represents the highest energy portion of solar radiation which reaches earth and numerous studies have thus been done on the potential effects of increased UVB radiation on plant growth and development. These trials have shown that high doses of UVB can cause damage to DNA and cell membranes, lead to protein degradation, impede photosynthesis and plant growth, alter pigment synthesis and interfere with the reproductive mechanisms (Caasi-Lit et al., 1997; Jansen et al., 1998; Jordan et al., 1992; Quaite et al., 1992; Zlatev et al., 2012). More recent studies have however revealed regulatory roles of low-fluence rates of UVB radiation (Heijde and Ulm, 2012; Singh et al., 2017a; Tilbrook et al., 2013). Field-grown plants seldom show the phenotypes typically linked to UVB damage, but rather display acclimation responses under low ecologically relevant doses of UVB radiation (Alonso et al., 2015; Coffey et al., 2017; Sen Mandi, 2016; Singh et al., 2014). Furthermore, it has been revealed that moderate levels of UVB can act as a significant environmental signal in plants, regulating a number of developmental processes which ensure that plants remain healthy and functional (Hideg et al., 2013; Huché-Thélier et al., 2016; Li et al., 2013; Yin and Ulm, 2017).

The presence of a UVB induced pathway which activates various UVB protection and repair systems in plants was revealed several years ago. Kliebenstein et al. (2002) characterised an *Arabidopsis thaliana* mutant of UV resistance locus 8 (UVR8) that is hypersensitive to UVB radiation. Results of this study suggested that UVR8 was involved in UVB mediated flavonoid biosynthesis and therefore plant defence systems. Rizzini et al. (2011) later showed that UVR8 acts as a photoreceptor which ultimately results in the induction of several photomorphogenic responses, thereby aiding in plant acclimation to UVB. The identification of UVR8 as the UVB photoreceptor has significantly advanced our knowledge of UVB mediated signalling, gene expression and morphological and metabolic responses in plants (Bernula et al., 2017; Jenkins, 2017; Loyola et al., 2016; Yin and Ulm, 2017). Examples include the production of antioxidants; accumulation of UVB absorbing compounds (Csepregi et al., 2017; Favory et al., 2009) and changes in leaf development (Fina et al., 2017). The UVR8 photoreceptor is present in fruit as well (Liu et al., 2015b) and a number of UVB mediated responses have been characterised in these tissues, most commonly the accumulation of UVB absorbing compounds such as anthocyanins and flavonols. This has been demonstrated in several fruits including tomato (Calvenzani et al., 2010), bell pepper (León-Chan et al., 2017), apple (Arakawa et al., 1985; Henry-Kirk et al., 2018; Ubi et al., 2006) and grape berries (Carbonell-Bejerano et al., 2014; Del-Castillo-Alonso et al., 2016; Liu et al., 2015b; Loyola et al., 2016; Martínez-Lüscher et al., 2014).

As a commercially important fruit crop, grapevine is extensively grown throughout the world. As such, it is exposed to a diversity of environmental conditions which influence grapevine growth and development. In addition, viticultural practices may influence the effects of environmental stresses and

it has become progressively more important to determine how grapevines perform under certain conditions. As a woody perennial, grapevine relies on the perception of the light environment to direct its seasonal progression and development as well as to ensure optimal light harvesting and mitigate any potential stress damage. Light furthermore modulates berry metabolites by influencing berry metabolic processes. Several light related studies have been conducted in grapevine to describe the effects on leaf physiology and composition (Dayer et al., 2017; Liakopoulos et al., 2006; Palliotti et al., 2000, 2009), photosynthesis (Bertamini and Nedunchezhian, 2002; Carvalho et al., 2016; Correia et al., 1990; Düring and Davtyan, 2002; Flexas et al., 2001; Palliotti et al., 2000; Smart et al., 1988), inflorescence formation and fruitfulness (Buttrose, 1969; Morgan et al., 1985; Srinivasan and Mullins, 1981) and berry characteristics (Chorti et al., 2010; Downey et al., 2008; du Plessis et al., 2017; Martin et al., 2016; Reshef et al., 2017; Smart, 1987; Song et al., 2015; Suklje et al., 2014; Sun et al., 2017; Young et al., 2016) . These trials have proven the significant influence of light on grapevine properties and many studies have extended these concepts to investigate the effects of particular spectral components of light on grapevine. Of increased interest has been the impacts of UVB radiation on grapevine, specifically in the Southern hemisphere which is known to receive higher levels of UVR. At the start of this study (in the 2013/2014 season), the information available on UVB mediated impacts in grapevine painted a somewhat incomplete picture, with limited trials being conducted in ecologically relevant settings. Several pertinent studies focusing on molecular and metabolic responses in grape berries have since been published, confirming that this study forms part of an international focus. These trials were conducted with the intention of better understanding the impacts of UVB, not from the perspective of damage, but more as a way to understand how grapevine organs respond and mitigate exposure to stress and how these responses relate to quality-impact factors in the different products.

Studies have shown that grapevine is in fact remarkably well adapted to environmental doses of UVB radiation and does not typically show stress responses (Jug and Rusjan, 2012; Martínez-Lüscher et al., 2013; Núñez-Olivera et al., 2006). Low-fluence rates of UVB radiation (~5.7 $kJ.m^{-2}$ at 30 latitude (Singh et al., 2017a) in field conditions have been demonstrated to elicit various acclimation responses in both the vegetative and reproductive tissues of grapevine plants, including changes in plant morphology (Berli et al., 2013a; Del-Castillo-Alonso et al., 2016; Doupis et al., 2016; Pollastrini et al., 2011), photosynthetic capacity (Alonso et al., 2015; Berli et al., 2013a; Doupis et al., 2016; Kolb et al., 2001; Martínez-Lüscher et al., 2013) and the metabolic profiles of leaves (Gil et al., 2012; Pontin et al., 2010a) and berries (Alonso et al., 2016; Del-Castillo-Alonso et al., 2016; Liu et al., 2015a; Martínez-Lüscher et al., 2014; Reshef et al., 2018; Song et al., 2015; Zhang et al., 2014). This ability to alter physical characteristics in order to acclimate to external environmental factors such as UVB radiation is called phenotypic plasticity. This method employed by plants is considered one of the most important to manage responses to environmental variability (Gratani and Loretta, 2014; Nicotra et al., 2010; Santo et al., 2013; Young et al., 2016).

Understanding the interactions between berry characteristics and the environment has been greatly advanced by the improvement in tools and technologies used to profile grapevine as well as characterize environmental conditions. The so-called "field-omics" concept (Alexandersson et al., 2014) aims to limit the effects of field variability by thoroughly characterizing the environment and crop growing conditions, thereby recognizing and quantifying potential factors that could be drivers in variability. The implementation of this type of approach has contributed significantly to the understanding of environmental impacts on grapevine systems in field settings. An integrated study of metabolomics data and micrometeorology, for example revealed the influence of variability in solar irradiance on spatial variations in cluster metabolic content and composition (Reshef et al., 2017). Modification of light quality and intensity integrated with micrometeorology and the metabolic composition of grapes and wine provided further insights into the development of major flavonoids in grapes and the resulting effects on the wine (Reshef et al., 2018). Grapevine field trials furthermore revealed the contributions of grapevine genotype and environmental factors and their interactions on the berry transcriptome, thereby providing a reference model from which to study how crop plants respond to their environment (Dal Santo et al., 2018). These studies demonstrated firstly, the importance of monitoring and characterization of environmental factors and secondly, the benefits of an integrated experimental approach, both of which contributed significantly to the understanding of grapevine function under field conditions.

1.2 The aims and objectives of this study

The aim of this study was to use a field-omics approach in an experiment where UVB exposure was modulated in a field setting, to evaluate the effects of both light quantity (exposure level) and quality (UVB attenuation) on metabolite modulation throughout berry development, as well as follow these impacts throughout the wine-making process.

The resources and motivation for this study are linked to two previous trials conducted in our environment in a model Sauvignon Blanc vineyard. In Young et al. (2016), manipulation of light exposure with leaf removal was validated over multiple seasons where the berry microclimate was shown to be altered in terms of light exposure to the bunch zone (light quantity). The study demonstrated that variations in visible light quantity were able to modulate changes in berry metabolite composition in order to mitigate stress related damage and these responses were dependant on developmental stage (Young et al., 2016). The second foundation study conducted in the same viticultural plot included a UVB radiation reduction treatment to investigate the effect of light quantity and quality on the composition and sensory profile of Sauvignon Blanc wine. Increased light exposure and UVB radiation significantly altered the chemical composition of the wines and also led to perceptible changes in the sensory attributes (Suklje et al., 2014).

The planned study took advantage of these results, using them as a foundation from which to conduct further experimentation and in particular, to extend the UVB studies by conducting a detailed analysis throughout berry development over three seasons. The validated high and low light microclimates of the Sauvignon Blanc vineyard provided the ability to investigate the influence of UVB on a white cultivar (Sauvignon Blanc) in both a high light and low light environment. Furthermore, the study was planned as a grapes-to-juice-to wine analysis to allow a more integrated understanding of the impact of exposure and UVB on the grape and wine matrices.

The following objectives were therefore established for the thesis:

1. To establish and validate a vineyard experiment, over multiple seasons, in order to study the impact of UVB exposure on Sauvignon Blanc berry development under both high light and low light microclimates.
2. To perform metabolite profiling of primary and secondary metabolites of the berries subjected to microclimates where exposure and UVB levels are modulated over the entire developmental season.
3. To evaluate the impacts of exposure and UVB on the transitioning of metabolites throughout the wine-making processes, including targeted chemical profiling of both juice and wine samples, as well as sensory analysis of the wines.

The data generated and outputs in the thesis will be presented as follows:

Objective 1 and 2 are addressed in Chapters 3 and 4

a. The previously characterized Sauvignon Blanc vineyard was utilized for the trial using the validated high light and low light experimental setup explained in Young et al. (2016) as a baseline.
b. Climatic data was recorded throughout the duration of the field experiment, to validate the treatments that are presented.
c. The sampling of the berries occurred throughout berry development to generate samples that cover the entire growing and ripening season.
d. Profiling of the changes in primary and secondary berry metabolites to follow their reaction to treatments throughout berry development is presented.

Objective 3 is addressed in Chapters 5 and 6

a. The juice profile in terms of grape berry composition and UVB modulation was evaluated.
b. The evaluation of targeted wine chemical data at different wine-making steps was conducted, including bottle-aged wines over two seasons;
c. Sensory evaluation of the wines and the linking of treatment factors to wine styles of Sauvignon Blanc made from the grapes subjected to different UVB and exposure levels was conducted.

The dissertation furthermore includes a concise literature review presented in Chapter 2 and is concluded in Chapter 7 with general insights and concluding remarks.

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Chapter 2

Literature Review

Light stress effects on grapevine organs and metabolism

Within their natural environments, plants are continually challenged by changes in their surroundings and have adapted numerous morphological and biochemical strategies to deal with these external abiotic stress factors. Grapevine, a widely planted and economically important fruit crop is no exception and in addition is known to display remarkable adaptability to a range of abiotic factors.

Most plants display mechanisms of stress tolerance, resistance and avoidance. The employment of these strategies not only ameliorates the potential for stress related damage but also allows for the optimisation of resource utilisation, ultimately ensuring the health and success of the plant. Studies investigating plant responses to stress factors are diverse and numerous and the term "stress" has been differentially defined by various such publications (Buchanan, 2000; Lichtenthaler, 1996). Taken together, stress has generally been defined as any detrimental condition which exerts an influence on plant growth, development and productivity. The plasticity of plant responses observed however means that plants are continually adapting to their immediate environmental conditions, in addition to transgenerational adaptations.

The stress concept and literature pertaining to the various aspects thereof has recently and expertly been summarised by Jansen & Potters (2017). In this review, the intention is to reiterate briefly the concept of stress in plants before presenting the known effects of light and specifically UVB stress on leaf and fruit responses, while summarising the extant knowledge of these effects in grapevine, focusing on berries.

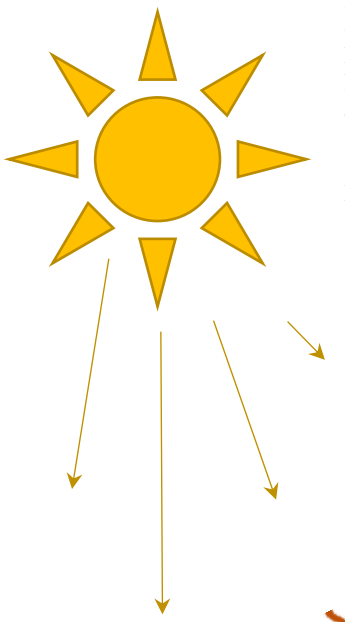
2.1 The concept of plant "stress"

Stress can be either positive or negative, depending on the end result. Lichtenthaler (1988) defined low levels of "adaptive" stress as "eustress" and high stress levels resulting in a negative outcome as "distress". Eustress drives the adaptive mechanisms employed by plants, optimising their state under the new environmental conditions, while distress results in damage to plant systems signifying their inability to successfully adapt (Hideg et al., 2013; Kranner et al., 2010). The definition of stress can therefore be extended to include the state of a plant where changing environments dictate an initial destabilisation of functionality, following by either successful adaptation/acclimation and improved tolerance, or damage and potentially death (Gaspar et al., 2002).

Adaptation refers to the long-term strategies employed by plants to survive in their environments. These include the evolution of special features through genetic mutations and natural selection over many generations. Acclimation on the other hand is the short-term response to external environmental stimuli which allows the plant to maintain optimal functioning without evoking any damage (Lichtenthaler, 1988, 1996).

2.2 Plant stress factors and responses

The inherent fluctuating nature of plant environmental conditions gives rise to a number of potential abiotic stress factors, to which plants have developed a variety of protective mechanisms. Plants have developed various ways of perceiving their environment and involve complex metabolic crosstalk within the multitude of plant biosynthetic pathways. Most stress responses occur at the cellular level which in turn lead to observable physiological symptoms. Following sensing of stress in plant tissues, the most appropriate defence response is initiated to manage or escape impending damage (Meena et al., 2017 and references therein). Examples of typical abiotic stress factors and responses are shown in Figure 2.1. Extensive research has been done on the effects of these main factors and a number of reviews exist which comprehensively summarise pertinent results and knowledge. Several examples of these studies and reviews are indicated in Figure 2.1.



Light
Increases reactive oxygen species (ROS) accumulation, disrupts photosynthesis, causes oxidation of proteins and lipids, decreases leaf area (Legner et al., 2014; Lichtenthaler et al., 1981), leads to thicker leaves (Lichtenthaler et al., 1981; Puglielli et al., 2017; Yang et al., 2014), elevated concentrations of chlorophyll and carotenoids (Calzavara et al., 2017; Lichtenthaler et al., 1981; Puglielli et al., 2017; Yang et al., 2014) and elongated palisade parenchyma tissues (Evans, 1999), induces adjustment of leaf angle (McMillen and McClendon, 1979; McNeil et al., 2016) and chloroplasts position (Dutta et al., 2017; Kasahara et al., 2002; Suetsugu et al., 2016).

UV-B radiation
Affects plant morphology (Caasi-Lit et al., 1997; Doupis et al., 2016; Tilbrook et al., 2013) impacts plant reproductive mechanisms (Jansen et al., 1998; Ries et al., 2000; Zlatev et al., 2012), promotes the generation of ROS (Hideg et al., 2013) and impacts photosynthesis (Jenkins, 2009; Kataria and Guruprasad, 2014; Zlatev et al., 2012).

Cold
Adversely affects plant growth and development and may lead to tissue breakdown and senescence (Chinnusamy et al., 2007; Thomashow, 2010).

Heat
Induces ROS accumulation (Belhadj Slimen et al., 2014; Das and Roychoudhury, 2014), enhances heat shock protein expression (Usman et al., 2014), reduces photosynthesis and respiration (Jajoo and Allakhverdiev, 2017), disrupts membrane stability (Xu et al., 2014), reduces seed germination, plant growth and development. (Iba, 2002; Wahid et al., 2007)

Drought
Affects plant growth and morphology (Anjum et al., 2011; Farooq et al., 2012; Jaleel et al., 2009; Nxele et al., 2017; Osakabe et al., 2014; Verslues, 2017), reduces stomatal conductance (Anjum et al., 2011; Arun-Chinnappa et al., 2017; Bota et al., 2016) impacts water use efficiency (Bota et al., 2016; Farooq et al., 2009, 2012), reduces photosynthetic capacity (Anjum et al., 2011; Escalona et al., 2003; Farooq et al., 2012; Osakabe et al., 2014), enhances ROS production (Arun-Chinnappa et al., 2017; Miller et al., 2010; Nxele et al., 2017; Osakabe et al., 2014; Zandalinas et al., 2017) and influences reproductive processes and organ formation (Barnabás et al., 2008; Farooq et al., 2017; Koshita and Takahara, 2004; Mahrookashani et al., 2017).

Salinity
Impacts plant morphology and growth (Munns and Termaat, 1986; Negrão et al., 2017; Parihar et al., 2015; Stavridou et al., 2017) induces stomatal closure (Munns and Termaat, 1986), reduces yield and leads to premature leaf senescence (Munns and Tester, 2008; Negrão et al., 2017), decreases water and nutrient uptake (Hussain et al., 2015; Negrão et al., 2017), induces oxidative stress (Hernández et al., 1995; Miller et al., 2010) and impacts photosynthesis (Chaves et al., 2009; Negrão et al., 2017).

Figure 2.1. The main abiotic stresses to which plants are typically exposed and the responses elicited by the different factors. The figure has been compiled using relevant publications as well as pertinent reviews.

To deal with these stresses and to mitigate any potential damage responses, plants have evolved a number of tolerance and avoidance mechanisms, enabling the plant to adapt and acclimate to the environment. Under water-restricted conditions for example, plants employ various morphological, physiological and molecular mechanisms which enable plants to delay or escape the detrimental effects of drought. These include the improvement of water uptake through the development of deep root systems, the minimisation of water loss through the development of smaller leaf areas, osmotic adjustment which facilitates the preservation of cell water balance through the active accumulation of solutes and the alteration of gene expression which aids in plant drought tolerance acquisition by, for example, regulating important proteins such as aquaporins and stress proteins (Farooq et al., 2009 and references therein). A further example is the employment of high temperature mitigation strategies, which may include avoidance or tolerance mechanisms. Avoidance mechanisms include morphological changes such as changes in leaf angle and leaf rolling, early plant maturation to avoid seasonal periods of high temperature, increased transpiration for cooling and stomatal closure to reduce water loss. Tolerance mechanisms include alterations to membrane lipid composition, reactive oxygen species (ROS) scavenging, antioxidant accumulation, signalling within plant systems, expression of heat shock proteins (Usman et al., 2014) and stress induced transcriptional changes. A number of reviews covering temperature responses in plants are available including those by Iba (2002), Chinnusamy, Zhu & Zhu. (2007), Wahid et al. (2007), Barnabás, Jäger & Fehér. (2008), Solanke & Sharma. (2008), Jajoo & Allakhverdiev (2017) and Ohama et al. (2017). Further implicated in the acclimation of plants to stress is the development of an epigenetic memory. This term encompasses the mitotically or meiotically heritable changes in gene activity which are not brought about by DNA sequence alterations. Epigenetic effects are involved in regulating transcription, DNA repair as well as regulating cellular responses to external environmental conditions. A number of stress responses, such as osmotic stress (Knight et al., 1998), oxidative stress (Knight et al., 1998), dehydration (Ding et al., 2014), excess light (Gordon et al., 2013) and temperature (Sung and Amasino, 2004) have elicited epigenetic marks in various plant species and tissues, aiding in their adaption and tolerance to the stresses (Annacondia et al., 2018; Bruce et al., 2007; Crisp et al., 2016; Hewezi, 2018; Muller-Xing et al., 2014 and references therein).

Several stress responses are common across the different abiotic stress factors; the most notable being the changes elicited in plant morphology and development, the impact on photosynthesis and the accumulation of ROS, all of which are inter-related. Photodamage is primarily caused by the oxidation of proteins, enzymes and lipids by ROS. The formation of ROS is inherent to the process of energy transfer and electron transport which is coupled to photosynthesis.

ROS induced damage occurs under conditions of high stress where the plant is unable to maintain the balance between ROS production and antioxidant defence. Under these conditions, increased ROS accumulation in cells may lead to oxidative membrane damage, protein oxidation, disruption of enzyme

activity, damage to DNA and RNA as well as the destruction of the cells due to oxidative stress. The activity of numerous detoxifying proteins and antioxidants present in plant cells help to mitigate these detrimental effects and include glutathione peroxidase (GPX), superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and peroxiredoxin (PRX) as well as ascorbic acid and glutathione (GSH) (Mittler et al., 2004). Certain metabolic processes also help to keep ROS levels in check through ROS detoxification. This antioxidant capacity is mediated by the activation of various pathways including the flavonoid, glutathione and phenylpropanoid pathways (Hideg et al., 2013). Plants have therefore evolved certain mechanisms to protect themselves from ROS induced damage. Under moderate stress conditions, when antioxidant systems ensure that ROS levels remain low, ROS may serve as signalling molecules which relay signals between plant tissue cells which activates certain acclimation processes including rapid adjustments to transcription and translation (Dietz et al., 2016). Specifically, ROS has been shown to be involved in signalling between the chloroplasts and nucleus of photosynthesising tissues (reviewed in Gollan, Tikkanen & Aro, 2015).

As an important commercial crop, stress responses in grapevine have received considerable attention (Cramer, 2010; Gerós et al., 2016). Studies have focused on specific individual factors including temperature (Arrizabalaga et al., 2018; Carbonell-Bejerano et al., 2013; Cohen et al., 2008; Luchaire et al., 2017; Mori et al., 2007; Pastore et al., 2017), light (Reshef et al., 2017, 2018; Sun et al., 2017; Young et al., 2016), water availability (Araujo et al., 2016; Griesser et al., 2015; Savoi et al., 2016) and salinity (Agaoglu et al., 2004; Stevens et al., 2011; Walker Read, P. E., and Blackmore, D. H., 2000) as well as in combination (Carvalho et al., 2016; De Oliveira et al., 2015; Doupis et al., 2016; Mirás-Avalos and Intrigliolo, 2017).

2.3 Light quantity and quality

Of the many abiotic factors to which a plant is exposed, sunlight is one of the most important as it is necessary for photosynthesis and various signalling processes integral to plant survival. The plant light environment is not constant and fluctuates with diurnal and seasonal changes and may also differ depending on climatic changes (e.g. cloud cover), shading by other plants and movement of leaves within a canopy. Furthermore, in agricultural situations, certain practices applied in the field such as trellising or directed leaf removal will lead to changes in the light environment. Additionally, light is composed of different components, each of which can elicit different responses in plants.

The perception of light quantity and quality provides vital information to the plant to allow for optimal growth and development. Examples include the perception of day length and therefore the season (Carr, 2001; Johnson et al., 1994), the determination of the direction of light incidence (Koller, 1986) and the provision of cues for internal circadian clock rhythms (Eriksson and Millar, 2003). Plants are also able

to utilise information on the light environment to adapt and acclimate to potentially stressful or adverse light conditions. Specific light mediated responses include seed germination, photomorphogenesis, phototropism, shade avoidance, flowering and senescence (reviewed in de Wit et al., 2016).

2.3.1 Components of light

The primary role of solar radiation is to drive photosynthesis whereby plants are able to harvest light energy and convert it into chemical energy to be utilized for various activities. Light however serves not only as an energy source, but also relays information to the plant through sensing and signally processes (Hernando et al., 2017; Kami et al., 2010). Perception of light incidence, intensity, quality and duration allow the plant to modify various processes and thereby ensure optimal growth and development. The solar radiation which reaches the earth's surface is made up of different spectral components, including infra-red, visible and ultraviolet light (Figure 2.2). In order to perceive their light environment, plants have evolved a diverse set of photoreceptors which detect specific wavelengths of light. These photoreceptors can be generally grouped into three different categories and include the phytochromes which absorb in the red and far red spectrum, the cryptochromes which are blue/UVA photoreceptors and include the phototrophins and Zeitlupes and finally the UVB absorbing receptor, UVR8 (Ballaré, 2014 and references therein; Galvão & Fankhauser, 2015 and references therein).

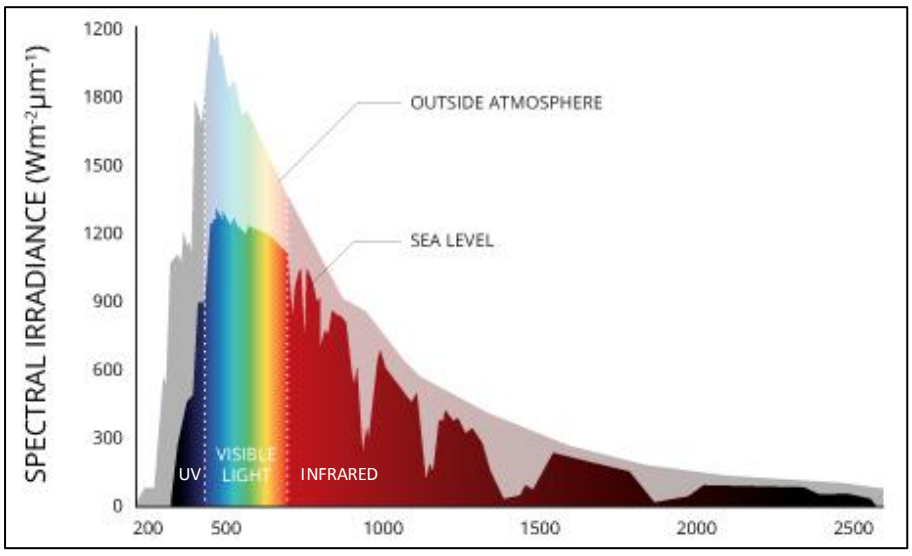


Figure 2.2. Spectral components of solar radiation reaching earth indicating ultraviolet radiation (UV), visible light and infrared. (Adapted from the image by Nate Christopher used with permission from Fondriest Environmental).

The ultraviolet light spectrum can be further sub-divided into three different categories, namely UVA, UVB and UVC (Figure 2.3). Having the shortest wavelength, UVC can potentially cause the most damage, however it is mostly absorbed in the atmosphere and very little reaches the earth's surface.

UVB is only partially absorbed by the atmosphere and this capability has been reduced in past years due to the depletion of the ozone layer. It has a short wavelength and therefore a high energy and has been shown to cause damage to living systems. These aspects have fuelled interest in UVB related studies and the effects thereof on living organisms. UVA is the most common form of ultraviolet radiation and is only absorbed by the atmosphere to a certain extent with most reaching the earth's surface (Diffey, 2002).

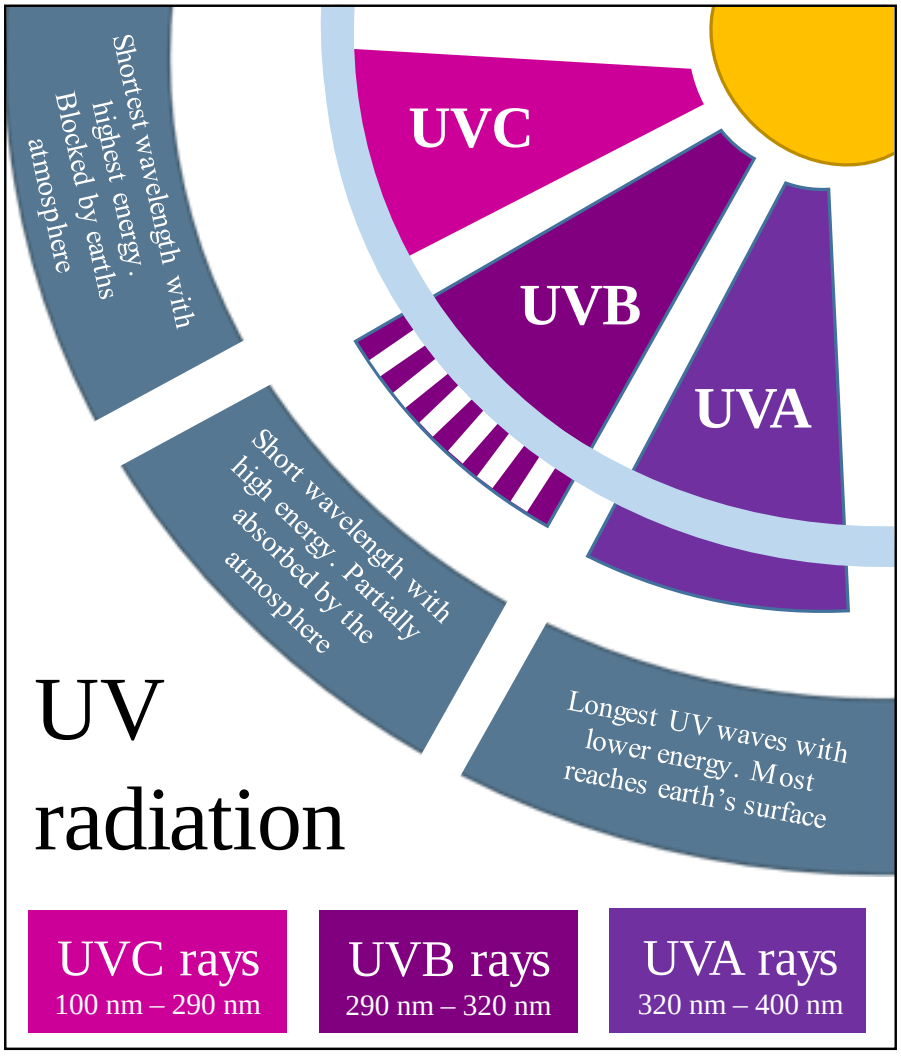


Figure 2.3. The different types of UV radiation spectral components

2.3.2 The utilisation of different spectral components and their impacts on plants

Studies looking at light as a stress factor and the mechanism employed to ameliorate damage are numerous. The type of stress response elicited by light will depend on the duration of exposure, the fluence rate and the predisposition for stress in the plant tissues. Light stress related studies have mostly been conducted in photosynthesising organs, mainly the leaves. The ability to adapt to variable light exposure which naturally occurs in an environment influences the photosynthetic efficiency of plants.

The capacity to absorb incoming solar radiation and transfer this energy to the reaction centres in the plant tissues under any light conditions is important. Plants utilize various acclimation strategies to regulate light harvesting and consequently strike a balance between energy utilization and dissipation, thereby limiting photo-oxidative damage. These strategies include several morphological, metabolic or transcriptional mechanisms.

Many light responses are induced by the synergistic and antagonistic actions of the different photoreceptors, which in turn regulate several aspects of plant functioning. It is therefore not only light quantity which is or significance, but also light quality.

The first identified light sensing molecules in plants were the phytochromes, which can be present in either the inactive or active form. The red light absorbing form (Pr) is the inactive form and is converted to the active far red absorbing form (Pfr) in response to red light. This active form is translocated to the nucleus of plant cells to trigger a cascade of signalling reactions. The equilibrium of these two phytochrome forms therefore regulate various plant processes including germination, flowering, photosynthesis and tolerance to biotic and abiotic stresses (reviewed in Demotes-Mainard et al., 2016). Exposure to different light conditions, including low light exposure, consequently uses this process to acclimate to the light environment.

Although the majority of research has been conducted on plant responses to red and far red light, blue and UV radiations are also significantly involved in photomorphogenesis and photosynthesis (reviewed in Huché-Thélier et al., 2016). For example, blue light can induce changes in shoot length, leaf area and dry mass, increase stem thickness, induce phototropism (de Wit et al., 2016) regulate chloroplast position within a cell and facilitate shade avoidance mechanisms (Franklin, 2016). Morphological responses to UVA have not received much attention; however, it has been shown that exposure to UVA affects leaf area, plant height and rosette diameter (Verdaguer et al., 2017). Blue and UV radiation have also been demonstrated to modulate metabolite accumulation and composition and stimulate various defensive mechanisms. For example, blue light exposure was shown to induce an increase in carotenoid content, while UVA tended to reduce levels. Furthermore, plants grown under blue and purple light demonstrated a higher incidence of phenylalanine ammonia lyase activity and therefore increased levels of flavonoids (Wang et al., 2010). UVA has also been shown to influence the accumulation of phenolic compounds in leaf tissues (Verdaguer et al., 2017).

UVB radiation though has been shown to mediate a number of morphological strategies which help mitigate potential stress responses. In grapevine, Doupis et al. (2016) demonstrated the ability to modulate leaf thickness depending on UVB dose. Furthermore, a comprehensive study by Berli et al. (2013) revealed a number of UVB triggered responses in grapevine leaves, including a reduction in

vegetative growth, most notably a reduced leaf area and an increase in leaf thickness. Similar results have been reposted in other plants, including increased leaf thickness, reduced leaf expansion, and the accumulation of cuticular waxes (Tevini and Teramura, 1989; Tilbrook et al., 2013). It has also been demonstrated that plants are able to adjust their leaf angle (McMillen and McClendon, 1979; McNeil et al., 2016) as well as the position of chloroplasts within a cell to ensure optimal light incidence without incurring any damage (Dutta et al., 2017; Kasahara et al., 2002; Suetsugu et al., 2016). Furthermore, UVB radiation has in several species been shown to stimulate an increase in flavonoid accumulation in plant epidermal cells as a way to protect tissues from damage (Calvenzani et al., 2010; Josuttis et al., 2010; Luthria et al., 2006; Mazza et al., 2000).

2.3.3 UVB perception and signalling pathways in plants

UVB radiation is intrinsically linked to light as it forms part of the solar spectrum. The depletion of the ozone layer has elevated concerns related to UVB induced responses in plant and the field has therefore received much attention. Plant responses to UVB are contextualised in terms of exposure dose which can be described as low (1 μmol $m^{-2}s^{-1}$) or high (1–3 μmol $m^{-2}s^{-1}$or above) dose radiation (Brown and Jenkins, 2008), as well as duration of exposure (Hideg et al., 2013). These factors will govern plant adaptation and acclimation or damage responses, thereby categorising the stress as either “distress” or eustress” as previously introduced.

UVB radiation represents the highest energy portion of solar radiation which reaches earth. Although linked to detrimental outcomes in plant tissues such DNA damage, protein degradation and photosynthesis disruption, UVB has been shown to induce photomorphogenesis and acclimation responses, thereby enabling the plant to tolerate UVB stress (Tilbrook 2013). Two general signalling pathways have been identified (refer to Figure 2.4), namely a non-specific signalling pathway which is stimulated by high levels of UVB radiation and a specific signalling pathway which is mediated by low fluence UVB radiation (Jenkins, 2009).

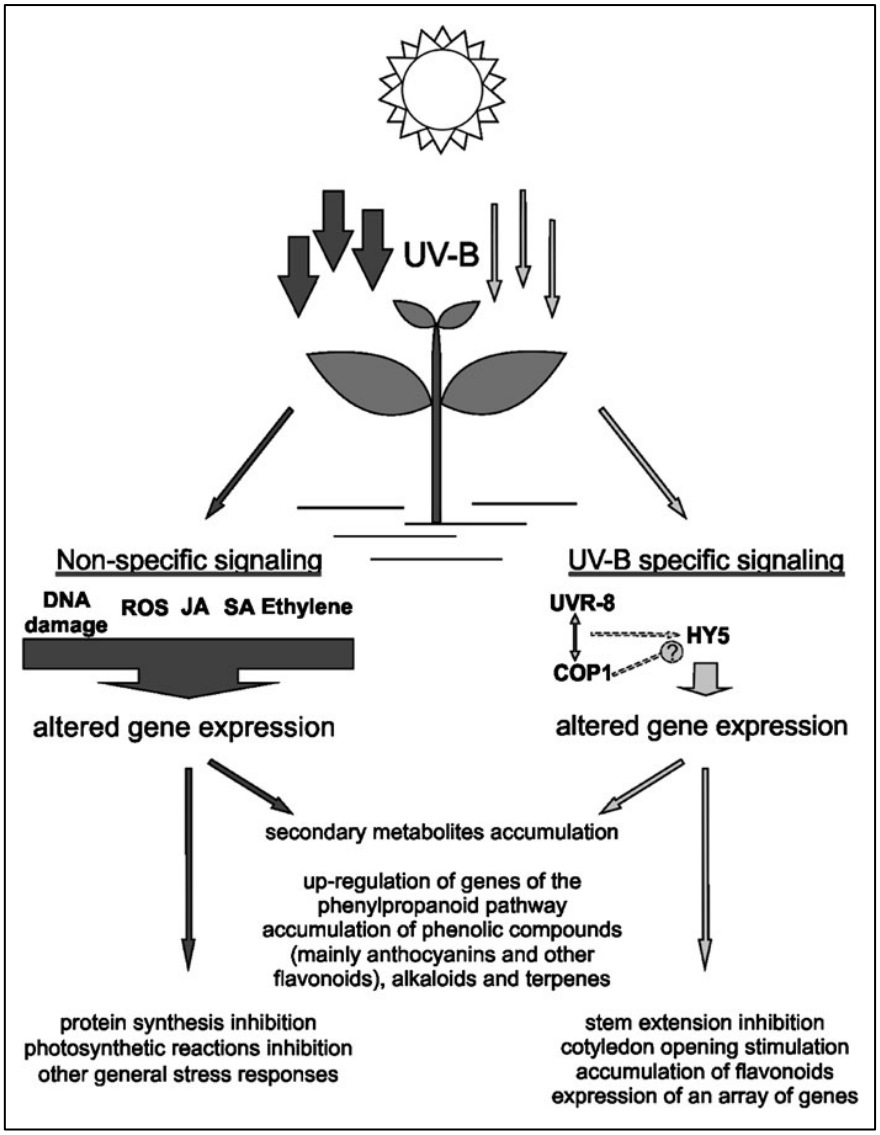


Figure 2.4. The main UVB induced non-specific and UVB-specific signalling pathways (Matsuura et al., 2013). The various abbreviations are defined in the text over the next two pages.

Related to non-specific signalling, a number of trials have demonstrated the damaging effects of UVB under high radiation levels, such as DNA damage, protein degradation, disruption of photosynthesis and stunting of plant growth (Caasi-Lit et al., 1997; Jansen et al., 1998; Jordan et al., 1992; Quaite et al., 1992; Zlatev et al., 2012). These responses constitute "distress" as UVB in these cases leads to disruption of plant metabolism, damage of cellular components and potentially cell or whole organism death. In response to high UVB radiation conditions, non-specific signalling stimulates the accumulation of secondary metabolites which may mitigate UVB related damage. This involves responses initiated by DNA damage signalling which induce a number of genes involved in UVB defence. Furthermore, UVB stimulates the accumulation of jasmonic acid (JA), ethylene, salicylic acid (SA) and ROS signalling compounds which are also involved in defence responses (Figure 2.4) (Jenkins, 2009; Matsuura et al., 2013 and references therein).

Despite the potentially damaging effects of UVB, it has become evident that under more "realistic conditions" where UVB adapted plants are exposed to ambient or near ambient levels of UVB radiation, "eustress" occurs. The response of plants to UVB radiation have therefore received particular interest, with more recent studies looking at the potential regulatory roles of low-fluence rates of UVB radiation under field conditions (Figure 2.4). These studies have shown that plants rarely display the classical UVB damage phenotypes, but rather show acclimation responses under low ecologically relevant doses of UVB radiation. It has been shown that moderate levels of UVB can serve as an environmental signal in plants, consequently regulating various developmental processes which ensure that plants remain healthy and functional (Hideg et al., 2013; Huché-Thélier et al., 2016; Li et al., 2013b; Yin and Ulm, 2017).

The presence of a UVB induced pathway which activates various UVB protection and repair systems in plants was revealed several years ago. Kliebenstein (2002) characterised an *Arabidopsis thaliana* mutant of UV resistance locus 8 (UVR8) hypersensitive to UVB radiation. Results of this study suggested that UVR8 was involved in UVB mediated flavonoid biosynthesis and therefore plant defence systems. Rizzini et al. (2011) later showed that UVR8 acts as a photoreceptor which ultimately results in the induction of several plant photomorphogenic responses to UVB.

UVR8 is different from other photoreceptors which use a prosthetic chromophore as it instead uses specific tryptophan amino acids for light absorption. UVR8 is constitutively expressed as an inactive dimer which is monomerised following the absorption of UVB radiation. The rapid accumulation of the active monomeric form of UVR8 occurs in the nucleus upon exposure to UVB. This consequently leads to the neutralisation of the salt bridges (connecting the UVR8 homodimers) causing the release of the active UVR8 monomers. These monomers associate with CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1) to form a UVR8-COP1 conjugate which activates the transcription of HY5 (ELONGATED HYPOCOTYL 5), a bZIP transcription factor that regulates a number of light responsive genes connected with photomorphogenesis and acclimation to UV (Reviewed in Favory et al., 2009; Jenkins, 2014; Yin & Ulm, 2017). Examples of the physiological roles elicited by UVR8 photoreceptor mediated UVB responses are presented below in Table 2.1.

Table 2.1. Selected physiological roles of UVR8-mediated UVB signalling.

Physiological roles of UVR8-mediated UVB signalling through various mechanisms as demonstrated in the model plant system, *Arabidopsis thaliana*		
UVR8 mediated role	**Mechanism of UVB impact**	**Reference**
Stomatal closure	UVB induced hydrogen peroxide (H_2O_2) and nitric oxide (NO), both of which are involved in stomatal closure. Evidence was provided for a mechanism involving H_2O_2 and NO generation which regulates stomatal closure through the UVR8 pathway.	(Tossi et al., 2014)
Leaf development	It was shown that UVR8 is involved in the regulation of the endocycle. UVR8 was also shown to stimulate stomatal development and lead to an increase in epidermal cell size in response to UVB.	(Wargent et al., 2009)
Inhibition of shade avoidance	The activation of UVR8 through the perception of UVB was shown to provide a signal which inhibited shade avoidance by blocking the synthesis of auxin and gibberellin which are phytohormones involved in plant growth.	(Hayes et al., 2014)
Inhibition of thermomorphogenesis	UVB perceived by UVR8 was shown to attenuate thermomorphogenesis through various mechanisms which inhibit the activity of the bHLH transcription factor PHYTOCHROME-INTERACTING FACTOR 4 (PIF4). UVR8 and the related COP1 mediated the repression of PIF4 transcript accumulation leading to a decrease in PIF4 quantity. Also, UVB stabilised the bHLH protein LONG HYPOCOTYL IN FAR RED (HFR1) which is capable of binding to PIF4 and thereby inhibiting its activity. Auxin biosynthesis is mediated by PIF4 which is necessary for thermomorphogenesis.	(Hayes et al., 2017)
Phototropism	A mechanism was proposed whereby UVR8 was able to influence directional bending towards UVB by inhibiting auxin signalling on the illuminated side, thereby reducing growth of this tissue.	(Vandenbussche et al., 2014)
Circadian entertainment	Low-intensity UVB (1.5 µmol $m^{-2}s^{-1}$) was able to act as an entraining signal for the circadian clock, a process which requires UVR8 and the associated COP1, HY5 and HY5 HOMOLOG (HYH). A model was proposed showing the interaction between UVB and the clock signalling pathways.	(Fehér et al., 2011)
Modulator of plant defence	Ecologically relevant doses of UVB radiation were shown to increase *Arabidopsis thaliana* resistance to *Botrytis cinerea*. It was demonstrated that UVR8 is capable of mediating the UVB induced pathogen	(Demkura and Ballaré, 2012)

	resistance by influencing the expression of the sinapate biosynthetic pathway.	
UVB acclimation and tolerance	Data shown in Favory et al. (2009) suggested a specific interaction between UVB and UVR8 and COP1 in early signalling consequently aiding in the elicitation of coordinated plant responses to UVB to ensure acclimation. Morales et al. (2013) further showed that UVR8 is necessary for the transcript accumulation of genes related to UV protection, oxidative stress, plant defence and hormonal signal transduction.	(Favory et al., 2009) (Morales et al., 2013)

These evident UVB responses and the interaction thereof with other environmental factors such as light quantity has made evident the need for evaluating plant system responses in relevant field conditions. Plants grown in the field will utilise the different photoreceptors in combination to perceive the variations in both light quantity and quality and respond accordingly by modulating the appropriate phenological processes. A significant body of work has been gathered utilising controlled lab conditions that have contributed to our detailed understanding of light perceiving mechanism and have increased our knowledge of light induced responses. However, there is a limitation to this approach in that extreme conditions are tested and these artificial conditions cannot accurately replicate the large fluctuations which occur in field settings. The interactions between different factors can also not be wholly simulated and neither is the long-term adaptability of the plant taken into consideration.

In a natural field setting, environmental fluctuations can induce the modification of a plant's characteristics as a way to adapt to these changes (Cramer et al., 2011). This ability is called phenotypic plasticity and can be defined as the capacity of a single genotype to express variable phenotypes when exposed to different environmental conditions (Nicotra et al., 2010; Gratani & Loretta, 2014 and references therein). This capability is considered to be one of the most important methods used by plants to manage environmental heterogeneity. In light of this, recent trials have rather been conducted under field conditions to investigate the effects of light quantity and quality on plant growth and development. Numerous approaches have been taken to carry out these field studies with a number of them being conducted on grapevine. A few examples are presented in Table 2.2. This summary highlights some important studies looking at the effects of UV in field conditions. The most typical UV response was the induced accumulation of UV absorbing or "sunscreening" compounds, mainly in the form of polyphenolic compounds. In the vegetative tissues UVB also affected plant growth characteristics. Considering the grape-related studies, the majority were conducted on red varietals with far fewer trials looking at the effects of UVB of white grape cultivars.

Table 2.2. A summary of the approaches taken in various trials to study the impacts of UV radiation on plants with their main findings.

Tissue type	Approach to investigating UV responses	Main findings	Reference
Plant leaves	Beech tree branches were encased in plastic filters which were either blocked or were transparent to UVB before budbreak at the start of the growing season.	Higher UVB levels reduced herbivory, reduced gallic acid concentration and increased flavonoid aglycone concentrations in leaves.	(Rousseaux et al., 2004)
	Soybean lines were grown under filters which transmitted different levels of UVB radiation. Frames were covered with either clear plastic which blocked all UV radiation below 310 nm or which transmitted most UV radiation.	UVB modulated leaf phenolics with the sunscreen response being induced by the UVB component of solar radiation.	(Mazza et al., 2000)
	Maize was grown under constructed wooden A-frames with plastics of different transmittance specifications draped over and stapled in place. The plastic sheets either absorbed UVB radiation or transmitted most solar radiation including UV.	UVB levels in solar radiation inhibited maize leaf growth without causing other stress symptoms. This was due to a reduction in cell production and shortened growth zone.	(Fina et al., 2017)
Grape leaves	The grapevine (cv Malbec) canopy was covered with a polyethylene plastic which transmitted variable degrees of UV radiation and photosynthetically active radiation (PAR). These were installed 2.5 m above the ground. The treatments were applied at different stages of development including 15 days before flowering, at flowering and early berry development and were maintained until harvest.	UVB induced UV absorbing compounds in leaves and increased accumulation of terpenes with antioxidant properties.	(Alonso et al., 2015)
	Grapevines (cv. Cabernet Sauvignon) were grown under polyester films which reduced UV light by 98%, while the control vines were left exposed to ambient conditions.	The total carotenoids were found to be less in leaves with reduced UV radiation.	(Steel and Keller, 2000)

	Filters were installed on either side of the canopy, covering the bunch zone and part of the canopy in cv Tempranillo. Different transparent polymetacrylate filters of varying UV transmittance were used, either blocking UV or allowing it through. These were installed pre-bloom and maintained until harvest.	Ambient UVB did not lead to stress symptoms in leaf tissues, but led to an accumulation of protective phenolics. Photosynthetic pigments were not affected, nor were there alterations in photosystem II (PSII) photochemical efficiency	(Del-Castillo-Alonso et al., 2016)
Fruit	In tomato, UVB radiation was modulated by covering entire growing tunnels with different materials including polyethylene films which allowed all solar radiation through and polyethylene films stabilised with the UVB absorber benzophenone in order to attenuate UVB reaching the plants.	UVB attenuation significantly reduced flavonoid content in tomato. The activation of flavonoid and light signal transduction genes was dependent on UVB, occurring mostly in mature fruit.	(Calvenzani et al., 2010) (Luthria et al., 2006)
	UV was modulated to strawberries grown in plastic tunnels by covering the tunnels in plastic films which either blocked UV or allowed it through. Open field strawberries were used as a control.	UV attenuation affected individual phenolic compounds. The content of the anthocyanin cyanidin 3-glucoside and the flavonols kaempferol 3-glucoside and quercetin 3-glucuronide were reduced with lowered UV.	(Josuttis et al., 2010)
Grape berry	Grape bunches (cv Cabernet Sauvignon) were encased in bags which absorb the UV components of sunlight shorter than 400 nm. These bags were composed of thick UV-proof film and only transmitted 0.04% of UV. Bunch clusters on the same vine were also enclosed in bags made of polyethylene film which transmitted sunlight including UV. These bags were installed at flowering.	In young berries, UV exclusion decreased the transcription of flavonol related genes thereby reducing the biosynthesis of flavonols.	(Koyama et al., 2012)
	The bunch zone (cv Sauvignon blanc) was covered with different transparent UV-transmitting/excluding materials mounted on a wooden A-frame. These sheets were composed of	Flavonols, specifically quercetin and kaempferol glycosides decreased with UVB attenuation with changes most evident in the skin. UVB was able to modulate various genes and	(Liu et al., 2015b); (Gregan et al., 2012)

	either acrylic (transmits all UV wavelengths), PETG (glycol-modified polyethylene terephthalate) (excludes UVB, transmits UVA) or polycarbonate (excludes all UV wavelengths). The treatments were carried out at 4 and 5 weeks prior to véraison.	transcription factors associated with flavonol biosynthesis. Methoxypyrazines and amino acids were not affected by UVB.	
	UVB radiation was modulated in the bunch zone (cv Malbec) by using polyester covers that absorbed UV to varying degrees. A second cover with different absorbance qualities was also used to create a near to ambient UVB environment. The treatments were applied 15 days after flowering.	High UVB led to increased phenolics in berries, specifically astilbin, quercetin and kaempferol and the Oxygen Radical Absorbance Capacity (ORAC).	(Alonso et al., 2016)
	Adjacent grapevines (cv Carignan and Grenache) were completely covered in plastic films of different transmittance properties to modulate UV radiation, while uncovered vines served as controls. These were applied at flowering.	The anthocyanin content of the skin was positively influenced by UV radiation which also modulated the different forms of anthocyanins, most notably cyanidin and peonidin derivatives.	(De Oliveira et al., 2015)
	The UVB in the bunch zone (cv Tempranillo) was blocked using transparent sheets installed at 45° from the vertical axis of the plant, on either side of the canopy. This was done pre-bloom (7 days before flowering).	UV induced secondary metabolism related transcripts including those involved in flavonol and monoterpenoid biosynthesis. Flavonol accumulation was most evident in skin tissues. Novel UV radiation-responsive transcription factors were identified. Also, UVB signalling pathway homologs were upregulated in berry skin by UV.	(Carbonell-Bejerano et al., 2014)

2.4 Metabolic responses to light modulation with pertinent examples in grapevine tissues

2.4.1 The accumulation of polyphenolic compounds in response to light and UVB

Phenolic compounds are produced through the phenylpropanoid pathway and its various related branches and has been characterised in a number of plants (Dixon et al., 2002; Ferrer et al., 2008; Hahlbrock and Scheel, 1989). In grapevine, the biosynthesis of polyphenolic compounds have received much attention due to their association with abiotic stress factors and the influence they have on wine attributes, including colour, astringency and mouthfeel. Their response to light has been well documented and the transcriptional mechanisms involved have also been characterised (Czemmel et al., 2017; Matus et al., 2009).

The most notable response to UVB in both the leaf and fruit tissues was an accumulation of polyphenolic compounds (Table 2.2). The accumulation of phenolic compounds can be related to the increased expression of various genes of the phenylpropanoid pathway which is involved in their biosynthesis in response to higher UVB radiation (Carbonell-Bejerano et al., 2014). The accumulation of these compounds has been demonstrated as a protective strategy against ROS (Castagna et al., 2017) and is mediated by the photoreceptor UVR8 (Favory et al., 2009; Morales et al., 2013; Stracke et al., 2010) under low fluence UVB exposure. These have been shown to elicit a "sunscreening" effect (Kolb et al., 2003; Landry et al., 1995a; Robberecht and Caldwell, 1983; Schmelzer et al., 1988; Xu et al., 2008) or serve as antioxidants which scavenge ROS (Agati et al., 2012; Carbonell-Bejerano et al., 2014; Hideg et al., 2013; Köhler et al., 2017).

Their ability to block harmful radiation lies in their chemical structure with their conjugated double bonds which absorb high energy wavelengths (Stevanato et al., 2014; Teixeira De Alencar Filho et al., 2016). The capacity to act as antioxidants is also related to their chemical structure and can occur in a number of ways. The hydroxyl groups of phenolic compounds can neutralize free radicals by accepting or donating electrons; following this interaction, a product is formed which is much more chemically stable. Their antioxidant capability may also be related to their capacity to chelate metal ions which are implicated in the development of free radicals. Furthermore, phenolic chemical structures are able to interact with proteins, enabling them to inhibit certain enzymes involved in the generation of radicals (Pereira et al., 2009). Extensive reviews on the biosynthesis and functionality of these compounds have been published in the past (Agati et al., 2012, 2013; Agati and Tattini, 2010; Brunetti et al., 2015; Hernández et al., 2009; Mouradov and Spangenberg, 2014).

The inducement of phenolic compound biosynthesis due to UVB has been demonstrated in grapes (Table 2.2). In a recent paper by Loyola et al. (2016) the grape UVR8 (VvUVR1) and HY5 (VvHY5 and VvHYH) orthologs were characterised and the role of UVB radiation in flavonol modulation was

demonstrated in grapevine leaves and berries. In the berries particularly, the activation of VvHY5 and VvHYH in the later developmental stages due to UVB favoured the accumulation of flavonols. Similar results were reported in Sauvignon Blanc by Liu et al. (2014) via the activation of VvMYB12 and VvHY5. Carbonell-Bejerano et al. (2014) furthermore demonstrated an upregulation of transcripts encoding for enzymes involved in phenolic biosynthesis with exposure to UV radiation. The main phenolic compounds involved were the hydroxycinnamic acids, the flavonols and the stilbenes. The UVB induced activation of MYB-related responses and the direct regulation of genes involved in the phenylpropanoid pathway definitively prove the effects of UVB on phenolic metabolism in grape berries. Similar response have been documented in other fruits (León-Chan et al., 2017; Arakawa et al., 1985; Ubi et al., 2006) and leaf tissues (Grifoni et al., 2016; Kolb et al., 2001; Pontin et al., 2010b).

2.4.2 The role of grape-derived terpenoids in UVB stress mitigation

Other UVB modulated pathways include those involved in stilbene and terpene synthesis. The antioxidant capacity of isoprenes has been validated in plants. Loreto et al. (2004) demonstrated the ability of isoprene to quench excess ozone in leaf tissues, thereby preserving photochemical efficiency as well as mesophyll structure, chloroplast envelopes and thylakoid grouping. Velikova et al. (2004) furthermore revealed the protective role of endogenous isoprene against singlet oxygen ($^1O_2$) and therefore oxidative stress. It was hypothesised that isoprenoids acted as scavenger molecules, taking up the excess energy and dissipating it as heat. These scavenger molecules are characterised by their conjugated double bonds which allow for easy energy transfer (Mittler, 2002). The localisation of these compounds in mainly the chloroplasts implicates them in the protection of photosynthetic machinery under high light and other stressful situations such as elevated temperature or UVB radiation (Logan et al., 2000). In grapevine leaves, low-fluence UVB has been shown to elevate membrane related triterpenes, suggesting a mechanism of acclimation, while high UVB elicited the accumulation of terpenes with antioxidant properties in mature leaves. The level of UVB exposure modulated metabolite accumulation in grapevine leaves. Monoterpenes and sesquiterpenes react readily with ROS (Calogirou et al., 1999), implicating them in oxidative stress mitigation. The regulation of terpene synthases (TPS) gene expression by UVB radiation has also been demonstrated in grapevine leaves, showing an upregulation under elevated UVB, leading to an increase in the biosynthesis of various terpenoid products (Gil et al., 2012; Pontin et al., 2010b). Other trials have also demonstrated an increase in certain aroma compounds in ripe grape berries (Bureau et al., 2000; Carbonell-Bejerano et al., 2014; Song et al., 2015; Zhang et al., 2014). Specific terpenoid biosynthetic genes were shown to be upregulated in the later developmental stages by increased UV radiation exposure (Carbonell-Bejerano et al., 2014). The stimulation of volatile organic compound accumulation with augmented UVB was also demonstrated in pre-harvest grapevine berries (*Vitis vinifera* L. cv. Malbec) by Gil et al. (2013). Sasaki et al. (2016) further demonstrated a reduction in the expression levels of genes responsible for linalool

biosynthesis with UV attenuation in Riesling (*Vitis vinifera* L.) berries. These volatile compounds are implicated in membrane stability, stress defence and antioxidant homeostasis.

2.4.3 The involvement of carotenoids in light stress mitigation

In grape berries, the aromatic C13-norisoprenoid compounds are degradation products of the carotenoids which are synthesised predominantly in the first stage of berry development when berries are photosynthetically active (Günata, 2013; Young et al., 2012; Yuan and Qian, 2016). The total concentration of carotenoids will be predominantly developmentally regulated; however, biosynthesis and accumulation can be affected by various environmental factors including light. Carotenoids are found in photosynthetically active tissues and are associated with Photosystem II where they serve as light harvesting antennae and aid in the prevention of photo-oxidative damage (Demmig-Adams and Adams, 2000; Demming-Adams and Adams, 1996; Nisar et al., 2015; Ruiz-Sola and Rodríguez-Concepción, 2012). The xanthophylls are carotenoids; secondary metabolites which occur in most plant organs and contribute to the red, orange and yellow pigmentation of flowers and fruits (Nisar et al., 2015).

The xanthophyll cycles are involved in alleviating light stress symptoms and several articles have reported on the capability of grapevine to develop and modulate the associated carotenoids for both the violaxanthin and lutein epoxide cycles (Düring and Davtyan, 2002; Razungles et al., 1996; Young et al., 2012). Young et al. (2012) furthermore reported on the presence of two isoforms of important enzymes involved in both xanthophyll cycles, namely zeaxanthin epoxidase (ZEP) and violaxanthin de-epoxidase (VDE). In higher plants, the xanthophyll cycles and PsbS protein have been identified as the main factors involved in energy dependant quenching (qE) (Ruban, 2016; Sacharz et al., 2017). These cycles are used in qE, a process that forms part of non-photochemical quenching (NPQ). The process of qE is dependent on the superfluous light energy the plant receives, consequently activating a signal which regulates light harvesting and elicits the protection of the photosynthetic machinery.

Depending on light conditions, either of two cycles may be activated to ensure optimal photosynthetic activity. Under high light conditions, the violaxanthin cycle is activated to form zeaxanthin. These molecules are able to take-up excess energy from the chlorophyll molecule and dissipate it safely as heat (Garcia-Plazaola et al., 2007). In sustained deep shade situations, the slow accumulation of lutein epoxide results in an increased light harvesting efficiency (Garcia-Plazaola et al., 2007; Matsubara et al., 2011). Exposure to sunlight will lead to the rapid formation of lutein, increasing the efficiency of photoprotection (Matsubara et al., 2006, Garcia-Plazaola et al., 2007). It has been suggested that lutein may be involved in photoprotection by facilitating energy dissipation. The combination of the

violaxanthin and lutein epoxide cycles therefore allow the integration of rapid and slow reversible reactions necessary for light harvesting and photo-protection (Garcia-Plazaola et al., 2007).

In green grapevine berries, Young et al. (2016) showed that increased light exposure in the bunch zone led to elevated levels of the carotenoids antheraxanthin and zeaxanthin in the green grape berries, while the chlorophyll a: chlorophyll b as well as the total carotene: chlorophyll ratios were conserved. It was concluded from these results that photosynthesis was maintained under increased light exposure due the protection provided by the xanthophylls. Similarly, in apple peel, increased light exposure led to elevated zeaxanthin levels, suggesting that the violaxanthin cycle was activated to prevent photo-oxidative damage (Cheng and Ma, 2004).

The involvement of these carotenoids in UVB protection has not received much attention, however, a few early studies have alluded to the carotenoids being related to UVB stress in certain plants. (Middleton and Teramura (1993) demonstrated an increase in photosynthetic pigments in response to UVB in soybean leaves and showed that the carotenoids specifically contributed to the photoprotection of photosystems. It was suggested that their protective capacity was related to their ability to quench high energy, short wave radiation. An earlier study hypothesised that this mechanism involved a photochemical state change of singlet oxygen to the triplet form by interacting with the carotenoids, thereby reducing the amount of oxygen radicals present which are produced during photo-oxidation (Krinsky, 1979). Another study conducted in the Cyanobacterium *Synechococcus*, observed a protective function of carotenoids in photosynthetic reactions against UVB. The effect was seen to be more pronounced in photosystem II activity with zeaxanthin being the most effective, implying a high protective potential of this carotenoid (Gotz et al., 1999). A mechanism of protection was suggested by Campos et al. (1991) whereby UVB increased the levels of hydroxy-3-methylglutaryl COA reductase mRNA which in turn induced the formation of carotenoids. A more recent study on tobacco (*Nicotiana tabacum L.*) leaves confirmed an increase in carotenoids with elevated UVB exposure and also showed a positive correlation with total antioxidant capacity (TAC). It was therefore suggested that carotenoids contain innate antioxidant abilities and their accumulation with UVB exposure could scavenge ROS under these conditions, thereby also protecting the plant from oxidative stress damage (Shen et al., 2017).

2.4.4 The role of certain amino acids in stress mitigation

Further implicated in acclimation and tolerance to UVB are the amino acids. The effects of UVB on grape related amino acids has not been extensively studied, however their roles in stress mitigation have been discussed in other plants with specific focus given to certain amino acids.

Preceding the discovery of the UVR8 photoreceptor, it was speculated that the aromatic amino acids in a protein which absorb in the UV range was involved in photoreception (Ballare et al., 1995; Ensminger, 1993). It has since been shown that UVR8 uses specific amino acid residues in the perception of UVB. UVR8 has been found to have 14 tryptophan residues, an amino acid which absorbs strongly in the UVB range. UVB exposure has been revealed to increase amino acid content in plants (Zu et al., 2004). This may be related to an amplified need for certain proteins, or increased protein turnover, specifically since proteins perform many important functions involved in stress tolerance and acclimation, most notably as enzymes, protective complexes and ROS scavenging compounds (Kosová et al., 2011). The metabolism of proline in particular and its involvement in abiotic stress damage mitigation has been extensively studied in plants (Anwar Hossain et al., 2014; Kavi-Kishor et al., 2005; Szabados and Savouré, 2010). These studies have also been extended to fruit and some information exists on its accumulation in these specific tissues in response to stress (Aihua and Mengyuan, 1989) (Yang, 1993) (Cao et al., 2012; Palma et al., 2014; Purvis, 1981; Wang et al., 2016b; Zhang et al., 2017). In grape berries, Berli & Bottini (2013) demonstrated an increase in proline in response to elevated UVB radiation. Another example of significant importance in stress related responses is the amino acid y-aminobytyrate (GABA). This amino acid is present in numerous plants and accumulates in response to various biotic and abiotic stresses. The increased interest in GABA and the GABA shunt pathway in plants arose primarily from the notable increases in GABA in response to stress. It has since been connected to a number of physiological reactions, including protection against oxidative stress (Bouché and Fromm, 2004). Numerous papers and reviews have been published outlining the role of GABA as a metabolite and its involvement in stress tolerance and adaption (Bouché and Fromm, 2004; Fait et al., 2008; Shelp et al., 1999, 2012).

Although the metabolic role of GABA in plant stress mitigation has been well characterised, a signalling role has long been speculated and research into this possibility has been conducted over the last two decades. Kinnersley and Turano (2000) initially observed the presence of plant GABA receptors in duckweed (*Lemna minor* L.) and a signalling role was first described by Palanivelu et al. (2003) where it was involved in pollen tube growth and guidance. Recently, a family of plant anion channels were discovered, namely the aluminium (Al3+)-activated Malate Transporters (ALMTs), which were shown to be regulated by GABA, confirming its role as a plant signalling molecule (Ramesh et al., 2015). A comprehensive review has since been published on GABA signalling in plants (Ramesh et al., 2017).

2.5 Conclusion

Berry growth and metabolism remains an important aspect to investigate from the perspective of quality-driven production. The changing environment will impact grapevine growth and development, depending on the severity of the changes in climate. Significant efforts are going into understanding the specific impacts that climatic factors could have on the grapevine biology, as expressed in growth and development of grapevine organs, however scope still exists for further investigation. Light quantity and quality modify various aspects of plant functioning with many responses aiding in acclimation to the environment. The many studies in grapevine have established its plasticity in response to many abiotic factors, including light. Metabolically, a number of pathways have been shown to be influenced by light leading to the modulation of several compounds including the polyphenolics, isoprenoids, photosynthetic pigments and amino acids. Recent focus has shifted to the different spectral components of light and their specific impacts on plant and grapevine metabolism. UVB has received particular attention due to concerns of damage and plant dysfunction. It has however been established that under field conditions, plants are able to adapt to environmentally relevant doses of UVB though various mechanisms, including the modulation of certain metabolites.

This study endeavoured to explore not only the impacts of light quantity, but light quality on berry metabolites, thereby identifying UVB-specific responses on berry processes and metabolites and distinguishing them from those responses elicited by variations in light incidence. Studies conducted on grapevine in particular have shown UVB radiation to be capable of altering the grape berry composition, however most studies have focused on red grape varietals, specifically at the ripe stage. Although these studies have shown that the presence or absence of UV-light may diminish or amplify specific compounds in plants, limited information is available on the impacts it may have on the underlying biochemical and metabolic processes involved. Here were attempted to provide further insights into the metabolic processes and their responses to UVB in a white grape cultivar (Sauvignon Blanc) throughout berry development.

Furthermore, in grapevine, the metabolites synthesised in response to light quantity and quality may all potentially impact the organoleptic properties of the wine. Most trials have however focussed on leaves or ripe berry tissue, specifically in red cultivars. This leaves significant scope to explore the effects of UVB, on white cultivars and in particular to try and understand it from the berry microclimate perspective. These impacts would be best understood if followed during the major berry developmental stages. In addition, novel information could be obtained if the UVB impacts on the berries could be followed through the wine-making steps to correlate compositional shifts of the juice and wine matrices to UVB impacts.

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Chapter 3

Field-grown grapevine berries use carotenoids and the associated xanthophyll cycles to acclimate to UV exposure differentially in high and low light (shade) conditions

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3.1 Abstract

Light quantity and quality modulate grapevine development and influence berry metabolic processes. Here we studied light as an information signal for developing and ripening grape berries. A *Vitis vinifera* Sauvignon Blanc field experiment was used to identify the impacts of UVB on core metabolic processes in the berries under both high and low light microclimates. The primary objective was therefore to identify UVB-specific responses on berry processes and metabolites and distinguish them from those responses elicited by variations in light incidence. Canopy manipulation at the bunch zone via early leaf removal, combined with UVB-excluding acrylic sheets installed over the bunch zones resulted in four bunch microclimates: (1) high light (control); (2) low light (control); (3) high light with UVB attenuation and (4) low light with UVB attenuation. Metabolite profiles of three berry developmental stages showed predictable changes to known UV-responsive compound classes in a typical UV acclimation (versus UV damage) response. Interestingly, the berries employed carotenoids and the associated xanthophyll cycles to acclimate to UV exposure and the berry responses differed between high and low light conditions, particularly in the developmental stages where berries are still photosynthetically active. The developmental stage of the berries was an important factor to consider in interpreting the data. The green berries responded to the different exposure and/or UVB attenuation signals with metabolites that indicate that the berries actively managed its metabolism in relation to the exposure levels, displaying metabolic plasticity in the photosynthesis-related metabolites. Core processes such as photosynthesis, photo-inhibition and acclimation were maintained by differentially modulating metabolites under the four treatments. Ripe berries also responded metabolically to the light quality and quantity, but mostly formed compounds (volatiles and polyphenols) that have direct antioxidant and/or “sunscreening” abilities. The data presented for the green berries and those for the ripe berries conform to what is known for UVB and/or light stress in young, active leaves and older, senescing tissues respectively and provide scope for further evaluation of the sink/source status of fruits in relation to photosignalling and/or stress management.

3.2 Introduction

Plants not only use solar light to drive photosynthesis and energy production, they also use it as a source of information about their environment. New information regarding the impact of the different spectral components of solar light (visible, UVA and UVB) are emerging, causing paradigm shifts with regards to the interpretation of existing and new results, the methods of experimentation, as well as the development of hypothesis and models to understand the intricate modulating effects versus the stress responses evoked by light components (Hideg et al., 2013). In the study of UV effects, it is now established that under ecological/field conditions, plants rarely display the classical UV damage phenotypes that have been established. Instead, a more complex picture is emerging showing that low ecologically relevant doses of UV are used by plants to acclimate and to modulate core processes to remain productive and thriving (Hideg et al., 2013; Li et al 2013).

UVB (280–315 nm) is an intrinsic part of solar radiation and is no longer considered a generic abiotic stress factor, but has been demonstrated to be a specific modulator. This is supported by the fact that UVB radiation is required for photomorphogenic responses (including acclimation) and is essential in the formation of the UVB photoreceptor, UVR8. In the absence of UVB radiation, UVR8 occurs as an inactive dimer (homo-dimers connected by salt bridges). UVB radiation causes a rapid accumulation of the active monomeric form of UVR8 in the nucleus, where the protein directly binds chromatin via histones. UVB radiation neutralizes the salt bridges (connecting the UVR8 homodimers) resulting in the release of the active UVR8 monomers. The UVR8 monomers subsequently conjugate with COP1, and this UVR8-COP1 conjugate activates the transcription of HY5. HY5, a bZIP transcription factor, subsequently regulates numerous light-responsive genes (>100 in *Arabidopsis*) involved in photomorphogenesis (Favory et al., 2009). In the absence of UVB radiation, UVR8 monomer dimerization is catalyzed by WD40-repeat proteins RUP1 and RUP2 (in *Arabidopsis thaliana*). Photomorphogenic responses to UVB radiation in leaves include reduced leaf expansion, increased leaf thickness, accumulation of phenolic compounds (predominantly flavonoids) and cuticular waxes (Tilbrook et al., 2013). These responses are comprehensively described for a number of plant species and specifically in photosynthetic organs (predominantly leaves), but data from fruit acclimation suggest that fruit in the early developmental stages, when chloroplasts are still functionally photosynthesizing, react in much the same way as leaves (via photo-protective mechanisms with the purpose of maintaining photosynthesis) (Blanke and Lenz, 1989).

Grapes are fleshy fruits grown in temperate areas of the world where a large proportion of similar cultivated varieties are produced under vastly different environmental conditions. The different climatic zones in viticultural production areas have been extensively characterized, particularly considering the potential impacts of climate change on berry metabolism and consequent quality. The responses of

field-grown plants (including grapevine) to biotic and abiotic stress are complex. Plants are typically exposed to multiple stresses and their responses are dynamic and overlapping and are classified as elastic (reversible) or plastic (irreversible) responses (reviewed in Cramer et al., 2011). Changes in the environment necessitate the alteration of the plant's phenotype in order to adapt to external environmental factors. This is referred to as phenotypic plasticity and is deemed the foremost method employed by plants to cope with environmental changes. *Vitis vinifera* has been shown to display phenotypic plasticity under these diverse conditions, particularly evidenced in berry transcripts and metabolites (Dal Santo et al., 2013; Young et al., 2016).

The limited research on grapevine berries and UV exposure in natural settings have shown that cultivated varieties are relatively well adapted to ambient UV exposure and typically show acclimation and not UV stress responses. Similarly, studies on other fruits and crops have revealed that acclimation responses to natural UVB levels involve the production of UVB absorbing flavonoids and phenolics. It has been shown that in some instances these compounds can act as UVB screens directly (Kolb et al., 2003), whereas in other occasions and/or locations, the inherent antioxidant capacity of the same compounds rather contributes to acclimation responses (Carbonell-Bejerano et al., 2014). The current understanding of UV effects on grapevine organs conforms to what is known for other species, i.e. with regards to the regulating aspects of UV stimuli, the phenylpropanoid pathway has been strongly linked to UV exposure. The observation that the attenuation of UVB reduces the accumulation of UVB absorbing compounds is not unique to grapevine and has been shown in a number of other fruits, including apple (Arakawa et al., 1985; Ubi et al., 2006), tomato (Calvenzani et al., 2010) and blackcurrant (Huyskens-Keil et al., 2012).

Several studies have focused on UV effects on grapevine berries (Gregan et al., 2012; Gil et al., 2013; Carbonell Bejerano et al., 2014), with some reports on vegetative and/or whole plant physiological performance (Pontin et al., 2010; Martínez-Lüscher et al., 2013). It has been demonstrated that the flavonoid biosynthetic pathway is transcriptionally regulated by UVB radiation in the skin of berries (Downey et al., 2004; Carbonell-Bejerano et al., 2014). Interestingly, a recent study on Sauvignon Blanc berries under different light and UV regimes lends support to the notion that in grapevine berries the biosynthesis of flavonols are increased through the classical low fluence UVB response pathway (Tian et al., 2015). Moreover, in the ripe berry stages putative terpenoid biosynthetic genes encoding for linalool and eucalyptol were upregulated in *V. vinifera* L. cv. Tempranillo in response to UVB radiation (Carbonell-Bejerano et al., 2014). Although these studies have identified possible regulatory genes and stress pathways that could be involved in UVB stress/acclimation, significant gaps still exist in our understanding of the mechanisms (and biological drivers) behind the observed responses. Additional motivation exists to clarify the effects of UV and general solar radiation on berry (and fruits in general) composition, since it is accepted to impact berry and wine quality.

The hypothesis of this study was that under field conditions high/low photosynthetically active radiation (PAR) and high/low UV exposures contribute in different ways to the response of berries to solar exposure. Our primarily objective was to distinguish between UV and PAR-specific responses on berry metabolites. To this end we evaluated Sauvignon Blanc berries in a high-altitude (model/highly characterized) vineyard where an experimental system to study berry metabolism under low and high (PAR) light exposure in the bunch zones was validated previously (Young et al., 2016). It was reported that specific metabolites responded to increased solar exposure [PAR+UV=High Light (HL)] in a metabolically plastic pattern in a likely process of antioxidant homeostasis, involving different metabolites depending on the developmental stage of the berries and when compared to the low light (LL) control. This characterized HL and LL experimental system provided an excellent opportunity to evaluate the specific responses and/or contribution of UV exposure to the metabolic responses. UV exclusion sheets were used to attenuate UVB light exposure (>99% reduction) on the berries under these two light regimes. In the first two seasons of the study, we found a strong light (PAR) and UV effect on specific berry carotenoid pigments, prompting a comprehensive analysis of the carotenoids and their derivatives (norisoprenoids) in subsequent seasons. Apart from two earlier studies by Schultz et al (1998) (reporting total carotenoids and zeaxanthin in Riesling) and Steel and Keller (2000) (ß-carotene and lutein in Cabernet Sauvignon), the impact of UV exposure on the photosynthetic pigments in berries is still relatively poorly described (compared to e.g. polyphenolics in red cultivars). Our results extend the current understanding of UV impacts in grapevine fruits (and fruits in general) by showing that specific carotenoids involved in photoprotection are responsive to levels of solar radiation (exposure), but that the UVB component in this light signal is required for the typical photo-protective response linked to the violaxanthin cycle under high light, as well as the accumulation of lutein epoxide under low light conditions. The ripe berry stages in particular displayed the accumulation of volatile compounds, but the profiles and levels depended on the specific level of exposure and UVB presence/absence. The results are discussed within the context of fruit metabolism in reaction to light as a source of information to modulate core processes.

3.3 Materials and Methods

3.3.1 Vineyard treatment, experimental design and berry sampling

A model *Vitis vinifera* L. cv. Sauvignon Blanc vineyard established in a commercial vineyard situated in the Elgin area of South Africa was used for the experiment. The vines were orientated in a north-west, south-east row direction and trained on a vertical shoot positioned (VSP) trellis system. Spur pruning to two buds was employed during winter and diligent canopy management occurred throughout the growing season. No water constraints were noted due to the high moisture content of the deep shale

soils, as was confirmed by stem water potential measurements in the same vineyard and reported in Young et al. (2016).

The experimental plot included three rows from which 16 panels were selected. Two controls and two treatments were applied randomly over the 16 panels with each control/treatment being repeated four times. Each panel consisted of four consecutive vines and represented a single biological repeat (Supplementary Figure 3.1 shows a diagram of the plot layout as well as images of the treatments).

Canopy manipulation via basal leaf and lateral shoot removal in the bunch zone (30-40 cm above the cordon) resulted in an altered exposure of the grape berries to light, thereby creating two distinctive bunch microclimates (with reference to exposure). This was done only on the East-facing side of the canopy, namely the side which was exposed to sunlight in the morning. A full characterization of the leaf removal treatment was recently reported in Young et al. (2016) that delivered a validated exposed versus a shaded bunch microclimate. UV light manipulation was achieved by installing UV-excluding acrylic sheets (Perspex® South Africa) over the bunch zone. The following four scenarios were therefore created in the vineyard: 1) complete leaf and lateral shoot removal in the bunch zone (30 to 40 cm above the cordon) on the morning side of the canopy (East side), generating the High Light control (HLcontrol); 2) a similar scenario to the first with the addition of a UVB excluding acrylic sheet installed over the bunch zone, generating the High Light-UVB (HL-UVB treatment); 3) no leaf or lateral shoot removal, constituting a fully shaded situation, generating the Low Light control (LLcontrol); 4) and a similar scenario to the third with the addition of a UVB excluding sheet over the bunch zone, generating the Low Light-UVB (LL-UVB) treatment.

Leaf and lateral removal as well as the installation of the UV-excluding sheets were carried out when the berries reached peppercorn size according to the Eichorn and Lorenz (EL) system (EL 29) (Eichorn and Lorenz, 1977). Sampling of the berries occurred at pea-sized berries (EL31), véraison (EL34), and ripe (corresponding to the harvest date; EL38) to yield samples that covered the full growing and ripening season. The stages corresponded to 26, 67 and 107 DAA (days after anthesis) in the 2011/2012 season and 25, 66 and 96 DAA in the 2014/2015 season. Berry sampling was carried out at each of the phenological stages on a per panel basis and therefore comprised of four biological repeats per treatment. Each sample consisted of 48 to 50 berries. Representative bunches on the exposed side (east-facing) of the canopy were selected from which to sample. Care was taken to select only berries from the exposed side of the selected bunches. Samples were frozen immediately after being picked in the field using liquid nitrogen and then transported to the laboratory. The seeds were removed and the remaining tissue milled in liquid nitrogen, after which they were stored at -80°C until analyses.

The trial was conducted over multiple seasons (2011/2012; 2013/2014; 2014/2015), but metabolite profiling mainly occurred in the first and last season and will be presented in the results section.

3.3.2 Climatic measurements

Climatic monitoring (meso-and micro-) occurred in the vineyard to quantify the main abiotic factors which could influence grapevine growth and development in response to the treatments. Various loggers and sensors were placed in the vineyard to measure climatic variables.

Temperature was measured at the mesoclimatic level via Tinytag® loggers (TinyTag Plus 2 - TGP-4500., Gemini Data Loggers (UK) Ltd., Chichester, United Kingdom) installed above the canopy. Similar loggers were placed within the canopy to measure temperature on a microclimatic scale. Bunch temperatures were monitored using dual channel temperature data loggers to which two thermistor flying lead probes were attached (TinyTag Plus 2 - TGP-4520). These probes were positioned within selected bunches from each of the controls and treatments. With regard to light measurements, both solar radiation (including PAR) and UV radiation were monitored. Solar radiation sensors (Vantage Pro2^{TM} solar radiation sensors Davis Instruments, California, USA) were also installed inside and outside the canopy. The outer unit measured the ambient solar radiation while the internal sensors measured the solar radiation which penetrated the canopy and reached the bunch zone. A solar sensor was placed in the bunch zone of each of the four light environments to determine the degree of light penetration in each case. UV radiation was measured using sensors (Apogee SU-100 UV sensors. Apogee Instruments Inc., Utah, USA) which were positioned similarly to the solar radiation sensors; one externally to measure ambient UV and one placed in the bunch zone of each created light environment. The solar and UV sensors were attached to two loggers (DataTaker DT82E data logger, Thermo Fisher Scientific Australia Pty Ltd, Victoria, Australia) which recorded measurements throughout berry development.

3.3.3 Analysis of major sugars and organic acid concentrations

The major sugars and organic acids of the berries were extracted and analysed using HPLC as described in Eyeghe-Bickong et al. (2012).

3.3.4 Analysis of photosynthetic pigment concentrations

The carotenoids and chlorophylls of the berries were extracted and analysed using UPLC as described in Lashbrooke et al. (2012) and Young et al. (2016) respectively. The de-epoxidation state (DEPS) of the xanthophylls were calculated as (zeaxanthin+0.5antheraxanthin)/ (violaxanthin+zeaxanthin+antheraxanthin) as described in Thayer and Bjorkman (1990).

3.3.5 Analysis of volatile aroma compounds

All authentic standards for volatile analysis were purchased from Sigma Aldrich (Steinheim, Germany): 6-methyl-6-heptan-2-one, trans-2-hexanol, 2-octenal, d-anisol, trans-2-heptanal, geralnylacetone, eucalyptol, limonene, trans-linalool-oxide, cis-linalool-oxide, linalool, 4-terpeneol, citronellol, nerol, geraniol, β-damascenone, α-ionone, β-ionone and pseudo-ionone, β-damascone and α-terpineol). Tartaric acid, ascorbic acid, sodium chloride (NaCl), sodium azide (NaN_3) and methanol were also acquired from Sigma Aldrich. For extraction of volatiles from grape berry tissue, approximately 1 g of ground, frozen tissue was weighed into a 20 mL GC vial and 2 mL of tartaric acid buffer (2 $g.L^{-1}$ tartrate, 2.1 g. L^{-1} ascorbic acid and 0.8 mg.L-1 L^{-1} sodium azide; pH 3) was added to each vial. Volatiles were extracted by head space (HS) solid phase microextraction (SPME) using a 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fibre (2 cm grey fibre from Supelco, Bellefonte, Pennsylvania, USA) (Barros et al., 2012). Prior to use, the fibre was conditioned at 270°C for 60 min in the GC injection port according to the manufacturer's specifications.

The samples were equilibrated at 60°C for 5 min in a heating chamber (with constant agitation at 250 rpm). After equilibration, the SPME fibre was inserted through the vial septa and exposed to the sample at 60°C for 30 min with constant agitation at 250 rpm. The bound analytes were thermally desorbed from the fibre in the GC injection port. After desorption, the fibre was maintained for 20 min in the injection port for cleaning in order to prevent potential carryover between samples.

GC analysis was carried out on an Agilent 6890N gas chromatograph (Agilent, Palo Alto, CA) system coupled to a CTC CombiPal Analytics auto-sampler and an Agilent 5975B inert XL EI/CI MSD mass spectrometer detector through a transfer line. Analysis was done using a Zebron 7HG-G009-11 capillary column (30 m × 250 µm ID, 0.25 µm). Desorption of analytes from the SPME fibre was performed in the injection port at 250°C by pulsed splitless mode for 1 min. The purge flow was 30 mL.min-1 (for 2 min). The column operating head pressure was raised from 111 kPa to obtain a pulse pressure of 300 kPa for 1 min. Helium was used as carrier gas with a constant flow rate of 1 mL.min-1. The oven parameters were as follows: initial temperature of 40°C (2 min), a linear increase to a final temperature of 240°C (at a rate of 10°C.min-1), and the temperature was held at 240^0C for a final 2 min. The total run time was 28 min. The transfer line temperature was maintained at 250°C. The MS detector was operated in scan and selected ion monitoring (SIM) modes. The scan parameters were set ranging from 35 to 350 m/z. The dwell time for each ion in a group was set to 100 ms. The software used was MSD ChemStation (G1701-90057, Agilent).

For quantification, external standard calibration was done by plotting standard curves using the ratio of the peak area of each authentic standard relative to that of the internal standard, versus the standard concentration (See Supplementary Table 3.1 for calibration parameters). Volatiles in samples were identified according to their elution times and masses compared to those of the respective authentic standards and quantified using the calibration parameters. Compounds without available authentic standard were identified by matching their mass spectrum with the Wiley 275 mass spectral library (Wiley, New York) and quantified. The resulted concentrations in µg/L were then divided by the berry fresh weight and multiplied by the sample volume (2 mL) to obtain the content (in ng/g FW). The selected ions used for the integration of peak areas of the respective compounds of interest, their retention time on the Zebron column, and quantifier molecules are summarised in Supplementary Table 3.2.

3.3.6 Analysis of polyphenolics

Total polyphenolic acids were analysed by HPLC on an Agilent 1200 at the Oxidative Stress Research Centre, Cape Peninsula University of Technology, Bellville, South Africa.

3.3.7 Statistical analysis

The resulting datasets were evaluated statistically, and were subjected to multivariate data analyses to integrate the different data layers. Microsoft Excel and Statistica (version 12) were utilised for standard statistical analysis. The responses of the various compounds to the individual treatments were tested for significance using a pairwise t-test. Testing was conducted on a "per developmental stage" basis. The contrasts examined were separated into HL and LL comparisons, thereby allowing for the examination of the effects of UV in a high light environment [HLcontrol (HL+ambient UV) versus HL-UVB] as well as a LL environment [LLcontrol (LL+ ambient UV) versus LL-UVB. Analysis of variance (ANOVA) was conducted on those pairwise contrasts with a p-value of < 0.05. Linear models were fitted to the contrasts showing significant variation in order to visualise the actual concentrations of the relevant compounds during berry development. Similar testing was conducted on the climate data to identify the main treatment effect(s).

Furthermore, a repeated measures ANOVA was conducted on the data in order to rank the significance of each compound in response to the three main experimental factors (i.e. development, light exposure and UVB radiation) individually and in combination. A repeated measures ANOVA was used to test for potential cause-effect relationships between the measured compounds and the main experimental factors. The results of the ANOVA are reported as F-values. The higher the F-value is, the lower the p-

value, and the greater the significance will be. Fisher LSD Post Hoc tests were used to confirm which compounds reacted statistically significantly to the specified factors (adjusted p-value, q-value).

Multivariate data analysis was conducted using SIMCA (version 12.0.3.0 from MKS Data Analytics and Solutions). The data was analysed using orthogonal partial least squares – discriminant analysis (OPLS-DA) .These models are used to relate the data matrix (X, the measured metabolites) to a specified qualitative vector (Y, class, e.g. developmental stage, exposure or UV). The use of supervised OPLS-DA models assisted in the visualisation of the complex datasets which consisted of multiple variables and helped to identify putative correlations within the dataset. The score plots are related to the individual observations which are grouped into similar patterns. The corresponding loading plots are used to relate the observed patterns in the OPLS-DA to the measured variables. Coefficient plots are displayed here in lieu of loading plots as they give an indication of direction. The X-variables are scaled and centred and the regression coefficients displayed are related to these values, thereby allowing for the comparison between coefficients. The size of the coefficient factor gives an indication of how strongly the Y-variable (i.e. development, light exposure or UVB radiation) is correlated to each of the X-variables (i.e. metabolites) (BioPAT SIMCA user manual).

3.4 Results

3.4.1 Characterization of the microclimates in the canopy and bunch zones.

The characterisation of the vineyard was performed according to the field-omics approach as explained in Alexandersson et al (2014). Detailed monitoring was performed in the vineyard and the climatic data are summarised in Table 3.1, indicating that the targeted parameters for this study, namely solar radiation (including PAR) and UVB exposure significantly differed in the microclimates generated for this study (Figure 3.1 and Supplementary Figure 3.2).

Table 3.1. A characterization of all the microclimatic climatic data collected in the 2014/2015 season on the sampling days during the sampling window (09h00 – 11h00).

		HLcontrol	HL-UVB	LLcontrol	LL-UVB
EL-31	Canopy temperature (C°)	24.4[a]	23.4[a]	24.3[a]	24.2[a]
	Bunch temperature (C°)	25.2[a]	25.4[a]	24.1[b]	23.7[b]
	Solar radiation (W/m²)	643.8[a]	707.8[a]	86.0[b]	86.8[b]
	UV (W/m²)	6.5[a]	0.4[b]	0.7[c]	0.0d
	Humidity (%)	57.1[a]	48.5[b]	59.1[c]	60.7[c]
EL-35	Canopy temperature (C°)	23.4[a]	22.8[a]	23.6[a]	23.6[a]
	Bunch temperature (C°)	29.9[a]	29.8[a]	23.7[b]	23.[b]
	Solar radiation (W/m²)	998.7[a]	855.1[a]	201.3[b]	198.0[b]
	UV (W/m²)	8.6[a]	0.6[b]	0.8[c]	0.0d
	Humidity (%)	48.8[a]	39.0[b]	49.4[a]	53.0[c]
EL-38	Canopy temperature (C°)	19.0[a]	18.5[a]	19.0[a]	19.0[a]
	Bunch temperature (C°)	21.1[a]	22.0[a]	18.6[b]	18.7[b]
	Solar radiation (W/m²)	168.2[a]	156.7[a]	71.7[b]	68[b]
	UV (W/m²)	12.8[a]	0.2[b]	1.0[c]	0.0d
	Humidity (%)	71.9[a]	62.0[b]	68.6[c]	70.0[a]

The specifications of the acrylic sheets used stated that they would be able to block out 99% of UV light. This was confirmed by measuring the UV radiation behind and in front of the sheets. Further specification of these sheets can be seen in Figure 3.1A, indicating that the UV-excluding sheets would block UVB (280-315 nm) since it attenuated wavelengths between 280 and 350 nm. When evaluating the HL and LL environments separately, ANOVA plots furthermore showed that the HLcontrol and HL-UVB treatment (and similarly the LLcontrol and LL-UVB treatment) had similar solar radiation exposure levels, confirming that the UV-excluding sheets did not change the solar radiation further (Figure 3.1B). The data confirmed that the UV-excluding-sheets effectively attenuated UVB radiation reaching the bunch zone (Figure 3.1C).

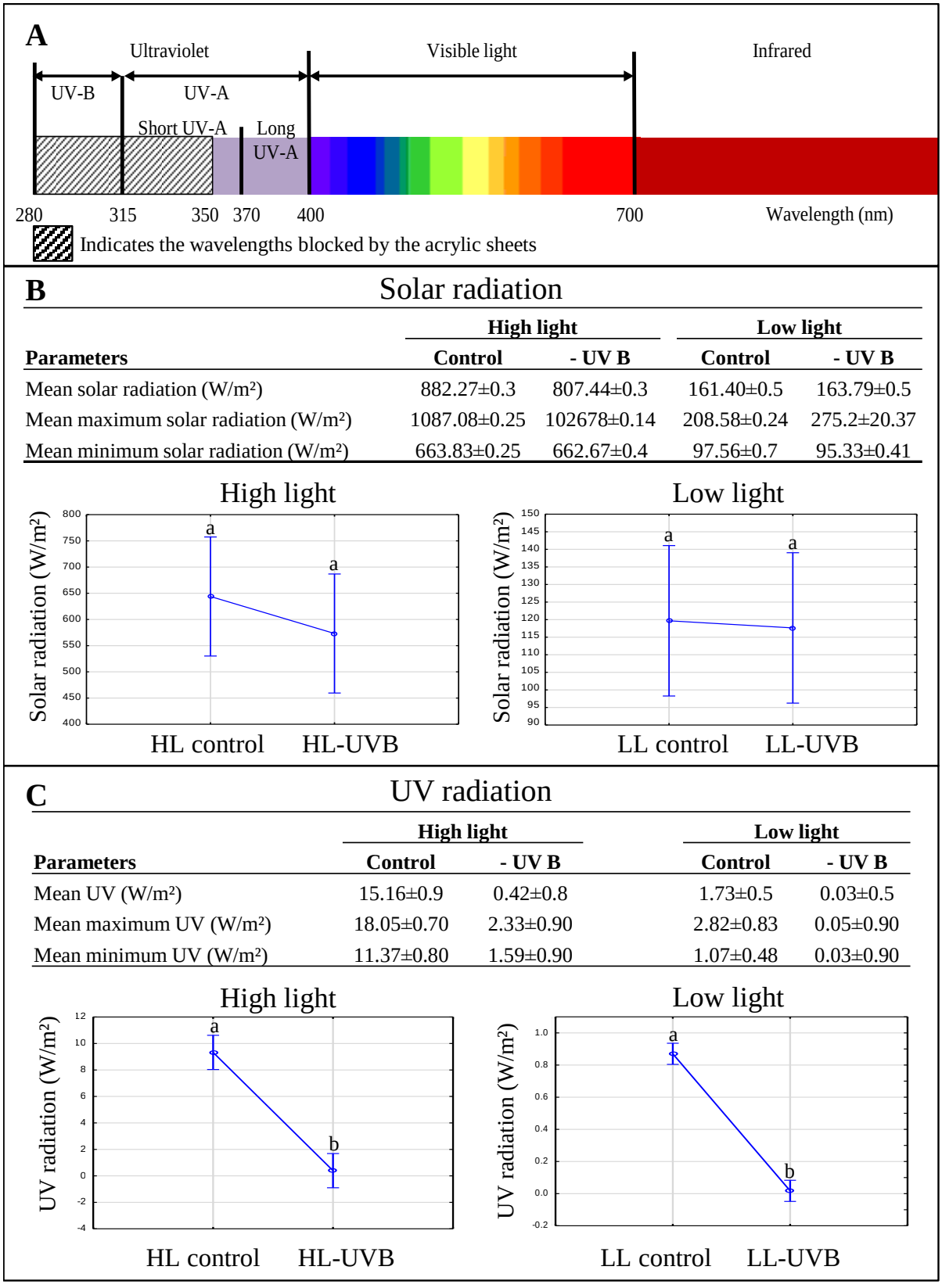
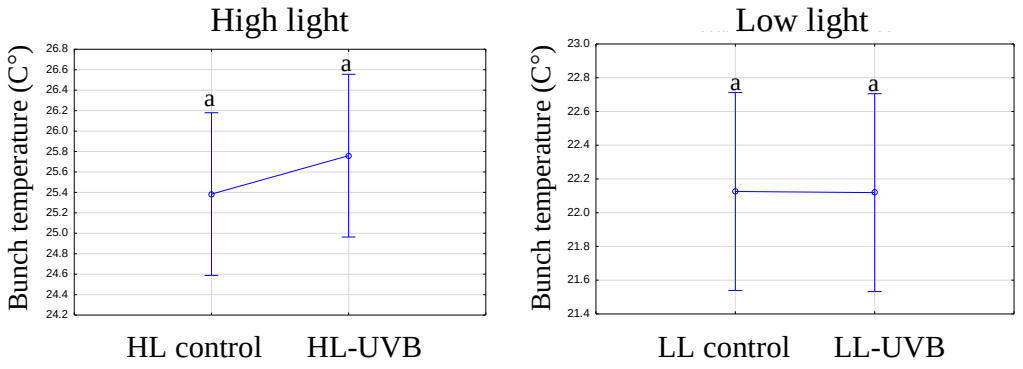


Figure 3.1. A characterization of the light microclimates created by the four treatments in the 2014/2015 season (A). The electromagnetic spectrum showing the wavelengths blocked by the acrylic sheets used in the experiment. (B) The mean(±SD), mean maximum(±SD) and mean minimum(±SD) bunch solar radiation values (B) and bunch UV radiation values (C) calculated for each light environment over the sampling window (9h00-11h00) and their corresponding ANOVA plots; different letters indicate significant difference ($p \leq 0.05$).

The leaf removal and increased exposure lead to differences in the bunch temperature between the HL and LL microclimates, but the UV-excluding sheets did not lead to additional differences in temperature within the HL (i.e. HLcontrol versus HL-UVB) or LL microclimates (Figure 3.2 and Supplementary Figure 3.3). The canopy temperatures were similar between all four the experimental scenarios.

A Bunch temperature

	High light		Low light	
Parameters	**Control**	**-UVB**	**Control**	**-UVB**
Mean temperature (C°)	25.38±0.09	25.76±0.11	22.15±0.07	22.12±0.09
Mean maximum temperature (C°)	27.72±0.14	28.04±0.12	23.94±0.11	24.04±0.11
Mean minimum temperature (C°)	22.68±0.15	23.37±0.16	19.98±0.16	19.91±0.15



B Canopy temperature

	High light		Low light	
Parameters	**Control**	**-UVB**	**Control**	**-UVB**
Mean temperature (C°)	22.27±0.05	21.8±0.05	22.33±0.05	22.25±0.05
Mean maximum temperature (C°)	25.26±0.10	25.27±0.10	23.96±0.09	24.03±0.11
Mean minimum temperature (C°)	22.41±0.12	22.22±0.12	20.81±0.13	20.66±0.13

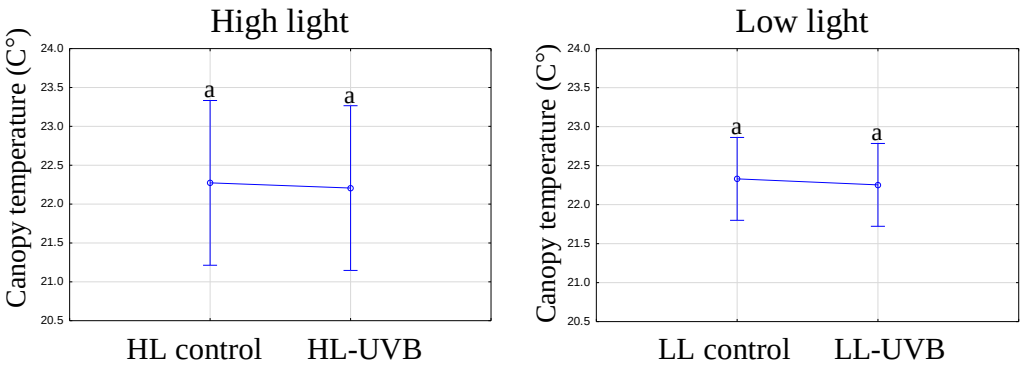


Figure 3.2. A characterization of the temperature data collected in each microclimate in the 2014/2015 season. The mean(±SD) mean maximum(±SD) and mean minimum(±SD) bunch (A) and canopy (B) temperatures measured on the sampling days during the sampling window (9h00 – 11h00) with the corresponding ANOVA plots for both high light and low light environments are shown; different letters indicate significant difference ($p \leq 0.05$).

3.4.2 Developmental and treatment impacts on berry metabolites

The ripening parameters showed typical developmental curves for grapevine berries (Supplementary Figure 3.4) with some variation in the total acids between seasons and samples at the earlier time-points.

When analysing the berry metabolites from the first season of study using a repeated measures ANOVA (Supplementary Table 3.3), developmental stage had the strongest effect on chlorophyll, carotenoid and xanthophyll pool sizes, and the latter two pools were also significantly affected by both the exposure of the berries, as well as UVB attenuation. These results prompted a more in-depth analysis in a subsequent season on the photosynthetically-related pigments, as well as volatile compounds in reaction to UVB attenuation. All the metabolite data measured over the two seasons in the green, véraison and ripe berries sampled from the four microclimates (HLcontrol, HL-UVB, LLcontrol, and LL-UVB) are provided in Supplementary Table 3.4.

OPLS-DA plots using developmental stage (Supplementary Figure 3.5A) or light exposure (Supplementary Figure 3.5B) as Y- variables, and the corresponding coefficient plots of compounds that contributed most to the models, highlighted metabolites that responded to the two factors. Separation in the samples was observed according to developmental stage with both primary and secondary metabolites contributing, in varying degrees, to the observed separation. Similarly, variation in light exposure also resulted in a clear separation between samples, confirming the influence of a high light and low light environment on berry metabolism (Supplementary Figure 3.5B). The metabolites mainly responsible for the separation, the xanthophylls, were similar to those previously reported by Young et al. (2016).

To better elucidate the subtle effects of UVB attenuation, OPLS-DA plots were created for the early and late stages of development separately. It was clear that different metabolites contributed to the separation in the green (Supplementary Figure 3.6A) versus ripe berries (Supplementary Figure 3.6B). The corresponding coefficient plots of compounds that contributed most to the models, highlighted specific xanthophylls and volatile aroma compounds that responded to UVB radiation/attenuation. The results of the OPLS-DA were further statistically validated by multifactor analysis (repeated measures ANOVA) in order to rank the significance of each compound in response to the three main experimental factors (i.e. development, light exposure and UVB radiation) individually, and in combination (Table 3.2).

Table 3.2. An analysis of the photosynthetic pigments and volatile aroma compounds (2014/2015 season). The repeated measures ANOVA results for the listed parameters and individual compounds are reported as F-values. Values are scaled from highest (i.e., most significant) to lowest by colour. Green indicates low F-values, while red indicates high F-values values. All insignificant values ($F \leq 3$) are coloured in grey. Maximum ■; 50% ■; minimum ■; insignificant ■

	Photosynthetic pigments						
	Development	Exposure	UVB-attenuation	Exposure × Development	UVB-attenuation × Development	UVB-attenuation × Exposure	UVB-attenuation × Exposure × Development
Total carotenoids	6969.66	108.26	0.11	143.63	2.91	1.64	3.76
Neoxanthin	5290.28	5.56	3.44	34.75	0.60	4.81	5.45
ß-Carotene+Lutein	4964.43	40.05	1.75	92.44	6.79	3.21	3.94
Chlorophyll b	4874.49	4.88	2.56	29.28	9.93	1.57	1.00
B-carotene	3946.08	39.94	2.88	56.21	4.89	3.64	3.90
Total xanthophylls	3532.44	207.91	24.29	143.68	1.79	0.79	0.07
Total chlorophylls	2844.18	7.81	1.03	24.82	3.92	0.65	0.35
Lutein	2407.50	18.29	0.00	110.95	4.99	0.86	0.40
Chlorophyll a	2203.64	8.62	0.63	22.16	2.49	0.41	0.21
Lutein epoxide	2019.62	163.13	29.35	131.01	4.88	24.76	40.76
Violaxanthin	1815.88	0.85	0.48	40.42	11.49	0.03	0.89
Carotene:Chlorophyll	1156.30	3.84	0.91	3.94	0.23	0.11	3.50
ß-carotene+Lutein: Total Carotenoids	733.40	98.97	3.85	60.23	2.87	4.69	1.83
Lx:L (ratio)	584.61	86.36	47.86	138.58	3.61	0.30	30.05
DEPS (ratio)	281.07	592.13	13.11	64.35	8.32	1.94	0.03
V+A+Z	161.48	250.02	22.85	72.91	7.14	17.08	4.92
Antheraxanthin	125.00	261.87	3.13	140.63	2.35	13.86	4.57
Chlorophylla: Chlorophyll b	114.44	54.70	2.90	11.46	0.11	12.99	7.32
Zeaxanthin	33.04	195.24	19.86	44.25	7.05	13.36	4.07
	Volatile compounds						
	Development	Exposure	UVB-attenuation	Exposure* Development	UVB-attenuation* Development	UVB-attenuation* Exposure	UVB-attenuation* Exposure* Development
b-Damascenone	1195.01	44.39	0.35	12.22	0.14	0.35	0.14
4-Terpineol	475.91	25.92	0.13	23.18	0.69	0.12	0.69
Mono-terpenes	438.47	37.45	0.05	7.69	1.37	0.03	1.28
a-terpinene	391.26	38.33	0.45	25.32	1.80	0.46	1.81
Geranylacetone	314.23	0.98	4.29	29.85	0.88	3.82	1.10
Cineol	299.63	0.03	0.39	0.03	0.39	0.39	0.39
Hotrienol	257.80	211.77	19.15	91.96	4.49	5.82	1.85
Norisoprenoids	238.93	1.49	6.26	33.02	3.97	2.79	1.03
Alcohols	234.48	207.44	15.19	89.72	4.21	3.43	2.79
Limonene	211.48	189.98	2.07	12.80	11.16	2.07	11.16
a-Terpinolene	205.19	66.92	0.08	23.89	1.22	0.05	1.23
Linalool	167.91	115.64	17.71	104.16	22.44	18.61	21.66
Sabinene	153.27	21.32	1.30	13.05	0.40	0.00	2.90
Gama-Terpinene	143.93	29.53	0.57	12.20	1.54	0.77	1.35
6-Methyl-6-heptan-2-one	84.04	0.00	10.61	71.19	14.54	0.29	14.25
a-Terpineol	62.55	104.88	6.36	23.13	3.13	6.36	3.13
1-Octen-3-one	60.04	36.70	25.00	2.26	14.63	28.84	35.30
Trans-2,4-Heptadienal	41.84	8.88	1.39	5.92	2.25	9.62	2.79
Geraniol	39.74	27.02	18.35	23.64	15.30	18.35	15.30
N-Hexanal	26.38	0.82	0.15	13.22	0.07	5.32	18.58
b-Cyclocitral	24.35	20.41	11.05	9.22	15.94	11.05	15.94
2-Octenal	23.74	90.77	20.74	15.30	8.02	11.14	26.33
Nonenal	22.77	2.68	2.73	2.99	0.46	0.74	6.33
2-Hexanal	16.86	1.07	6.97	1.71	3.05	0.65	2.22
Carbonyl compounds	14.49	0.00	1.69	0.83	1.03	0.04	4.66
Trans-2-Hexanal	12.63	0.03	2.17	0.74	0.98	0.36	3.63
Octanal	11.01	32.36	0.92	9.34	3.41	3.59	6.96

3-Hexanol	10.25	2.27	0.51	0.87	2.40	25.24	4.13
b-ionone	9.06	2.14	0.08	1.59	2.66	0.08	2.66
2-Heptanal	5.39	90.95	11.79	21.84	5.33	9.84	23.08

To simplify and visualise the data according to the main focus of the study ("What is the impact of UVB on berry metabolites and how is it different from exposure?"); compounds that responded to the variation in light exposure and/or UVB-attenuation were used to create Venn diagrams per developmental stage (Figure 3.3). Fisher LSD Post Hoc tests were used to identify statistically significant changes. Interestingly, in the pre-ripening stages, all compounds that responded to exposure, also responded to UVB attenuation. These compounds therefore differed in amplitude, and not in presence or absence. In the ripening stage, however, compounds were identified that responded only to UVB attenuation.

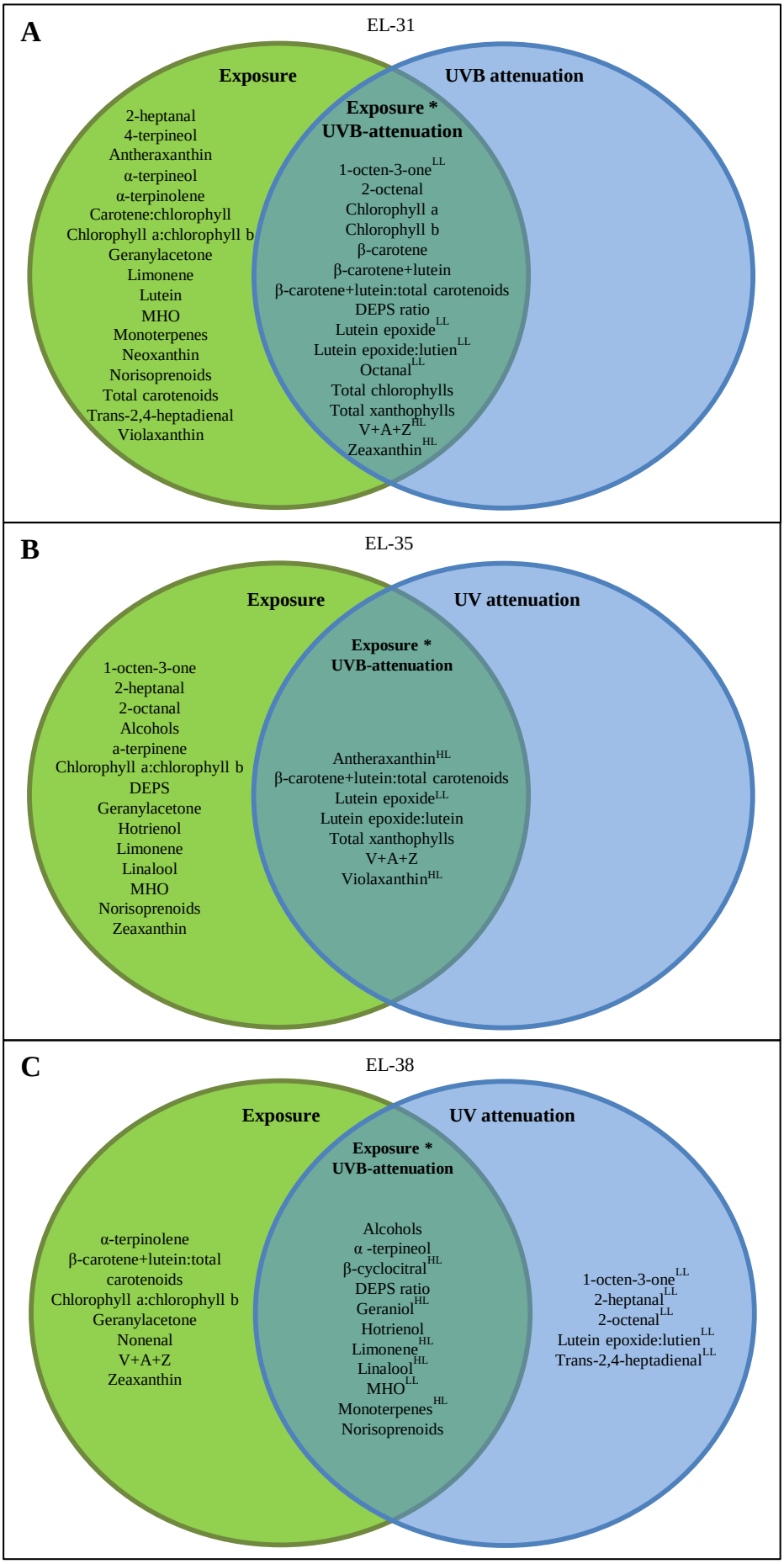


Figure 3.3. A Venn diagram showing the compounds which responded to light exposure (green circle), UVB attenuation (blue circle) and both (intercept) in the early (A), véraison (B), and late (C) developmental stages. Compounds were selected based on significance in a repeated measures ANOVA and Fisher LSD Post Hoc tests (adjusted p-value, q-value ≤0.05). All metabolites presented have a q-value ≤0.05. Metabolites with a log2-fold-change of ≥0.5 are indicated by a "HL" for the high light- or "LL" for the low light microclimate.

3.4.3 Specific xanthophylls responded to UVB attenuation in predominantly the green photosynthetically active berry stages

During the early stages of development, the xanthophylls zeaxanthin and lutein epoxide were identified as being the most responsive to UVB attenuation. Interestingly, the responses to UVB attenuation differed between the HL and LL environments. The attenuation of UVB in the HL environment resulted in a statistically significant decrease in zeaxanthin (Figure 3.4). This in turn resulted in a smaller xanthophyll pool size (violaxanthin, antheraxanthin, zeaxanthin) and a consequent lowered de-epoxidation state (DEPS ratio) in those samples (Figure 3.4). Although this was particularly obvious at the green berry stage, the lower xanthophyll pool, and consequent lower DEPS ratio, was consistently seen throughout berry development in the HL-UVB microclimate, but decreasing with developmental stage progression. Furthermore, the attenuation of UVB in the LL environment also resulted in a decreased V+A+Z pool and a lowered DEPS ratio in the green stage (Figure 3.4), although the effect was less pronounced compared to HL.

A significant difference in the levels of lutein epoxide between the LLcontrol and LL-UVB contrasts was also confirmed, clearly showing that UVB exposure in LL conditions is involved in the metabolism of lutein epoxide. Since lutein levels did not change, the Lx:L ratio was consequently significantly affected in the green developmental stage and to a lesser degree at the harvest stage (Figure 3.4).

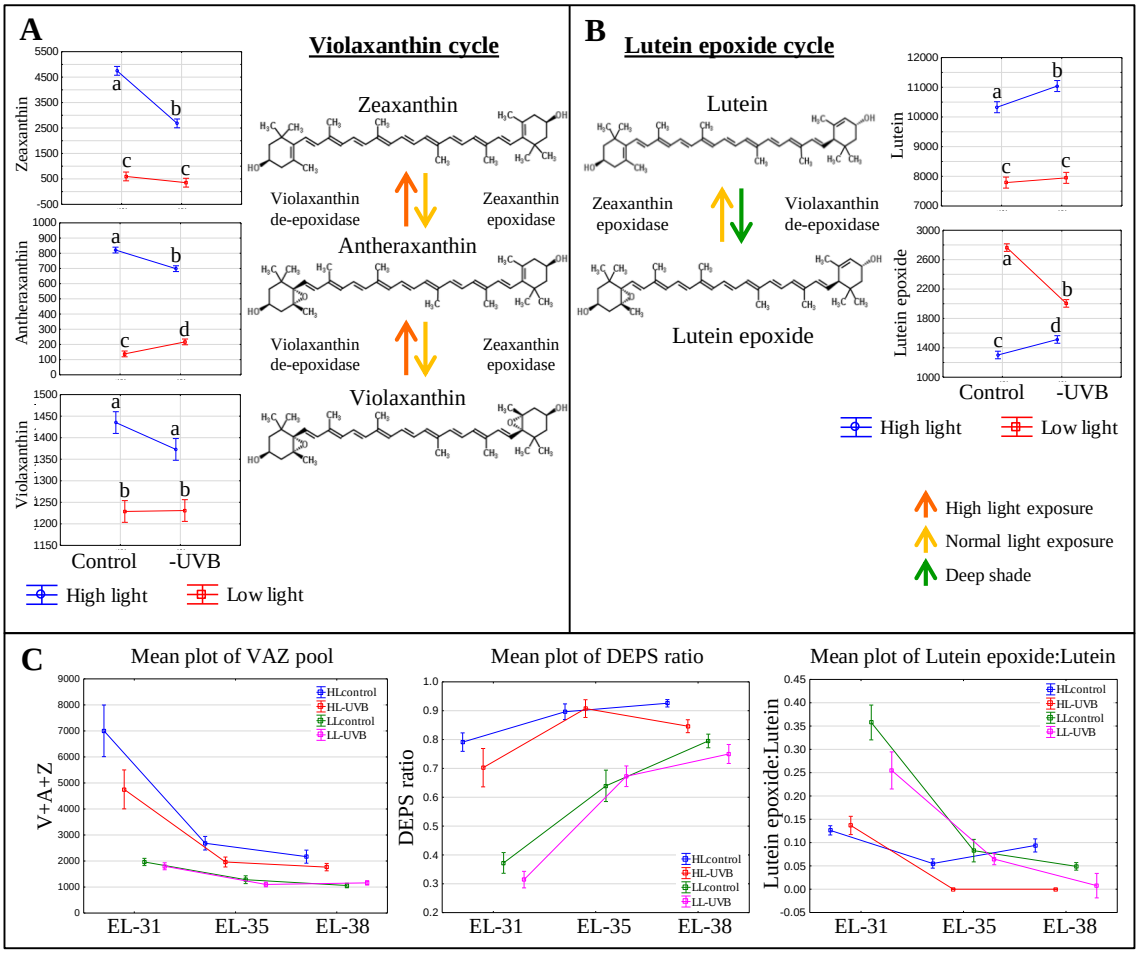


Figure 3.4. The violaxanthin (A) and lutein epoxide (B) cycles with the ANOVA results for their associated xanthophylls in the green developmental stage (EL-31). Different letters indicate significant difference ($p \leq 0.05$). The mean plots of the associated xanthophyll pool (V + A + Z), DEPS ratio and lutein epoxide:lutein ratio for both high- and low light environments over all developmental stages (C).

3.4.4 In the ripe berry stages specific volatiles responded to UVB attenuation

UVB attenuation was shown to affect specific volatile compounds in the ripe developmental stage (EL-38). These included monoterpenes, carotenoid-derived norisoprenoids and certain C_6 compounds. In the HL environment, certain monoterpenes and norisoprenoids were decreased by UVB attenuation, leading to larger monoterpene and norisoprenoid pools in the HL control samples (Figure 3.5) and confirming that UVB exposure stimulates volatile organic compounds (VOCs) in exposed berries. Under LL conditions, however, both the monoterpene and norisoprenoids pools were decreased relative to the HL microclimate and UVB attenuation resulted in no further statistically significant differences between the LLcontrol and LL-UVB microclimates.

Interestingly, under LL conditions, different VOC profiles as well as contents of individual volatile compounds were observed when comparing the LLcontrol with the UVB attenuated microclimate (LL-

UVB) in ripe berry samples. Certain straight chain aldehydes and ketones (e.g. 1-octen-3-one, 2-heptanal and trans-2,4-heptadienal), decreased with UVB attenuation. Conversely, a significantly higher concentration of C_6 compounds, including trans-2-hexenal and N-hexanal were observed when UVB was attenuated in the LL environment. This is the opposite of the scenario in HL, where the HLcontrol had more total C_6 compounds than the HL-UVB (Figure 3.5).

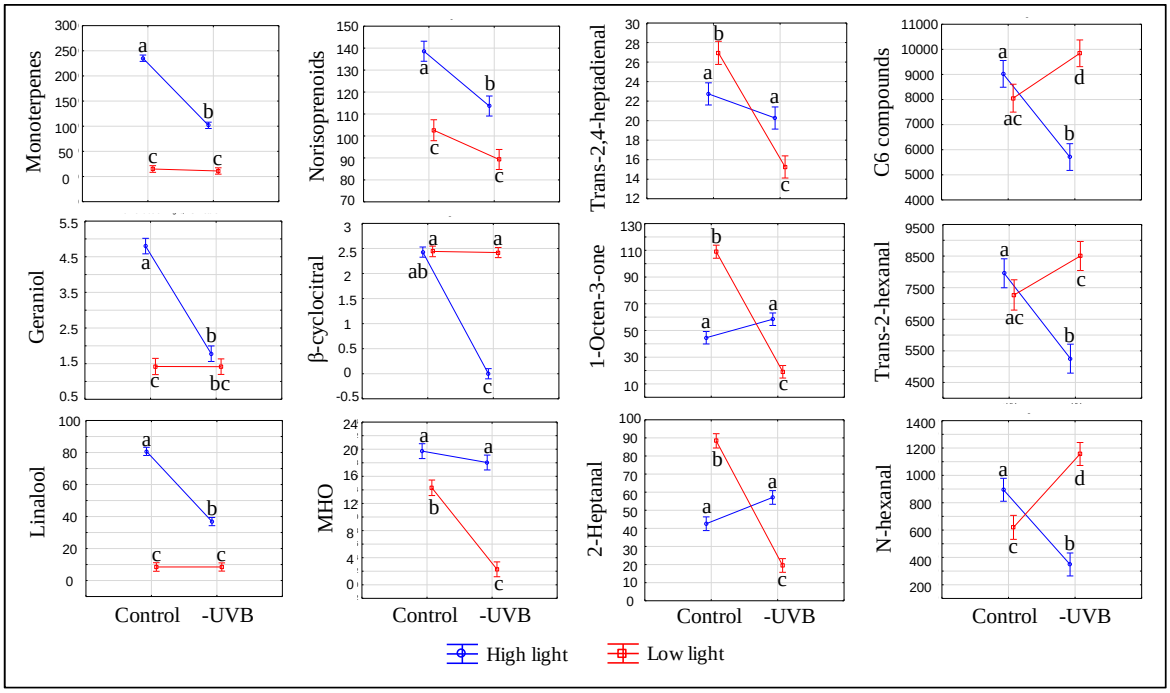


Figure 3.5. Analysis of variance (ANOVA) plots for selected volatile compounds, including monoterpenes, norisoprenoids and C6 compounds measured at the late developmental stage (EL-38). The results for both high light and low light environments are represented. Different letters indicate significant difference ($p \leq 0.05$).

Furthermore, to control for well-known metabolite responses to UV, samples were also analysed for polyphenols. As expected, total polyphenolics, and specifically the flavonol quercetin-glucoside, was significantly reduced with UVB attenuation in the HL microclimate, most notably in the early developmental stages (Figure 3.6A), although this pattern followed through to harvest (Figure 3.6B). No statistical significances were seen in the LL microclimate (LLcontrol versus LL-UVB) in either the early or late developmental stages.

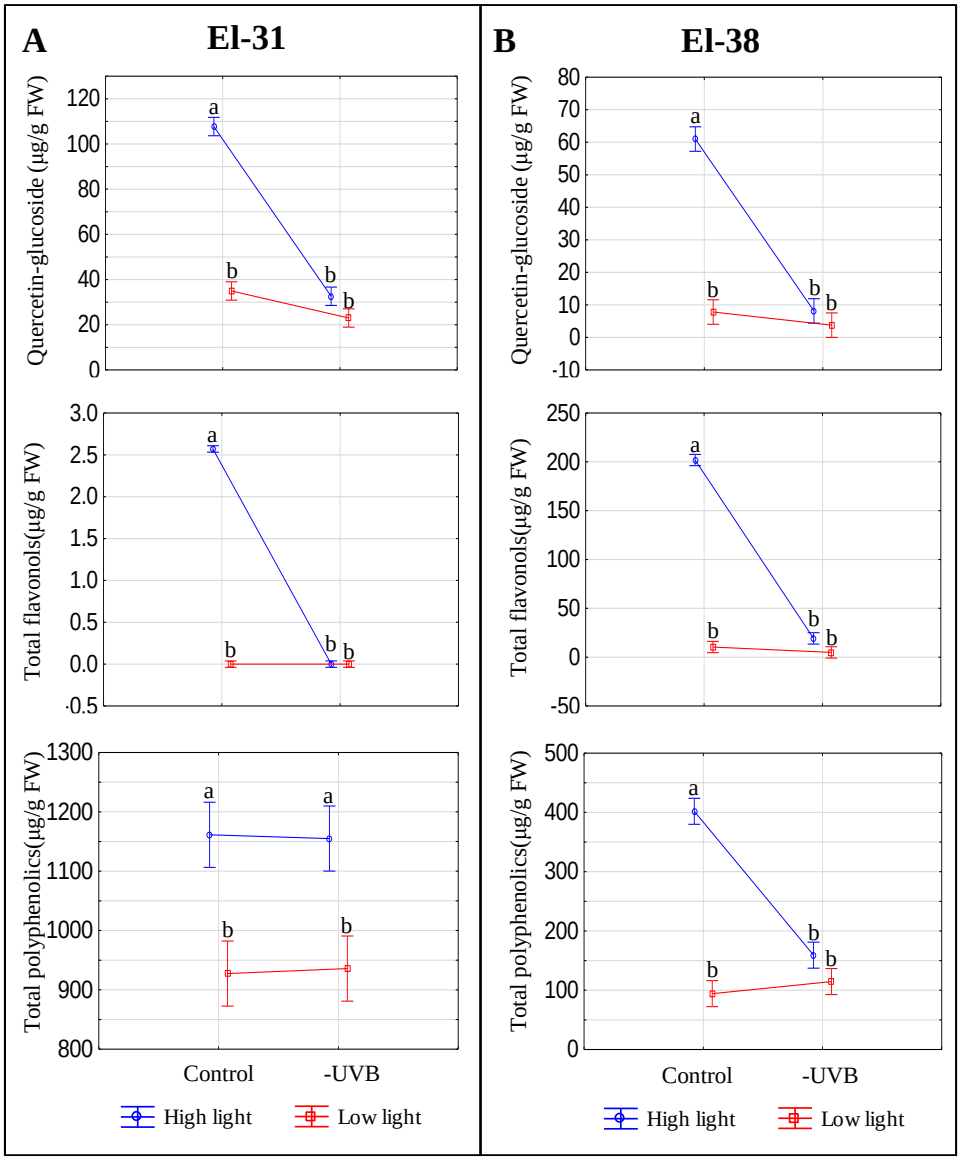


Figure 3.6. The ANOVA plots for quercetin-glucoside, total flavonols and total polyphenolics measured at the early (EL-31) (A) and late developmental stage (EL-38) (B). The results for both high light and low light environments are represented. Quercetin-glucoside is expressed in µg/g fresh weight. The pooled compounds are expressed relative to quercetin-glucoside (µg/g fresh weight). Different letters indicate significant differences ($p \leq 0.05$).

3.5 Discussion

A number of studies have shown that increased exposure (including UV) of grape berries, leads to the increased accumulation of polyphenolic compounds (Tardaguila et al., 2010; Diago et al., 2012; Song et al., 2015), as well as changes to varietal aroma compounds (Bureau et al., 2000; Zhang et al., 2014; Song et al., 2015). The increase in phenolic compounds, including anthocyanins, proanthocyanidins and flavonols, have been attributed to the increased expression of a number genes involved in their biosynthesis as a way to adapt to high light environments (Matus et al., 2009; Azuma et al., 2012). Carbonell-Bejerano et al., (2014) demonstrated that UV radiation upregulated a number of genes encoding transcription factors (e.g. MYBs and bHLH) that in turn activated flavonol biosynthetic genes (putative lyases, chalcone synthases, flavonol synthases (FLS) and flavonol glycosyltransferases) in grape berries. FLS is a dedicated enzyme involved in flavonol biosynthesis (e.g. quercetin) and its transcriptional response to light has been demonstrated in Shiraz (Downey et al., 2004).

In this study the characterization of the microclimates confirmed exposure and UVB attenuation as the main treatment effect in both the high and low light environments. Marked increases in quercetin-glucoside contributed to a higher content of total polyphenolics in ripe berries in the HLcontrol (compared to HL-UVB), but not in the LL microclimate (Figure 3.6). The study illustrates that grapevine berries utilize polyphenolics as well as photosynthesis-related pigments in acclimation responses. These responses are differentially affected by UVB attenuation under HL and LL conditions in the different berry developmental stages. Since the carotenoid pigments are substrates for the formation of volatile aroma compounds (norisoprenoids) as ripening progresses, these volatile berry metabolites were also followed.

3.5.1 Grapevine berries displayed metabolic plasticity in their response to attenuated UVB and the response was influenced by the developmental stage of the berries

In the green berry stage (EL-31) the xanthophylls reacted to the variations in UVB. This modulation of xanthophylls in the photosynthetically active green berries indicated that within the field setting, acclimation to light stress occurred in the early developmental stages. The data showed that the violaxanthin and the lutein epoxide cycles were functional in the photosynthetically active berries in the HL and LL microclimates. The amplitudes of the cycles were, however, responsive to solar radiation and UVB. Although these cycles appear to be functional in the photosynthetically active green berries, and are typically regarded as photo-protective measures, the major carotenoids and chlorophylls were not significantly affected ($\log_2$-fold change ≤ 0.5) in either microclimate (HL or LL). This implies that the stress perceived by the photosynthetically active berries in the early developmental stages was mitigated by, for e.g. photoprotective mechanisms (e.g. non-photochemical quenching via the violaxanthin cycle) and photosynthesis was apparently unaffected (i.e. no evidence of photoinhibition

and/or photodamage based on the core photosynthetic pigments). In the absence of UVB radiation, the berries required less zeaxanthin in HL microclimates, and conversely, less lutein epoxide in LL microclimates, to cope with the perceived stress and maintain active photosynthesis. The attenuation of UVB however potentially renders the plants more susceptible to damage as they are less acclimated than those plants exposed to UVB, especially in the low light microclimate. From numerous studies on photosynthetic organisms/tissues, it is known that the xanthophylls respond to light by way of the violaxanthin and/or lutein epoxide cycles (Demming-Adams 1996; Garcia-Plazaola et al., 2007).

The photosynthetic efficiency of plants depends on their ability to adapt to natural daily variations in photon flux density. It is important that the photosynthetic plant tissues are able to absorb solar light and transfer the resulting energy to the relevant reaction centres under any light conditions. The light environment within a canopy is not fixed, but fluctuates in occurrence with the creation of gaps in the canopy or climatic changes (e.g. cloud cover). The alterations in the light environment may be transitory (e.g. sunflecks), or more permanent (e.g. leaf removal). In response to the variations in light exposure, plants have developed several morphological, physiological and biochemical mechanisms to optimise the light harvesting process as well as to protect the photosystems and maintain optimal functioning (Walters and Horton., 1994; Demming-Adams and Adams., 2006; Johnson et al., 2007; Garcia-Plazaola et al., 2007; Vogelmann and Gorton., 2014). It is evident that berries have maintained this photoprotective ability and respond to stress in the same way as photosynthetically active leaves.

In the HL microclimate, UVB-exposure lead to increased production of berry volatiles (predominantly monoterpenes including geraniol, linalool and limonene with a $\log_2$-fold change >1) in the later stages of berry development (from véraison onwards). Similar results were seen in Malbec berries in that increased UVB exposure resulted in an increase in monoterpene emissions at the pre-harvest developmental stage. These results were interpreted to suggest that monoterpenes were involved in protection from UVB radiation (Gil et al., 2013). The antioxidant potential of terpenes (isoprene, monoterpenes, sesquiterpenes and tetraterpenes such as carotenoids) is well documented (Loreto and Velikova 2001; Loreto et al., 2004) and it is possible that this is one of their biological functions in older (sink) tissues (such as ripe berries and/or senescing tissues).

A similar result was seen in the norisoprenoids in the HL environment with the most responsive of them being β-cyclocitral. In a LL environment, MHO was seen to react in a similar way in that it was significantly reduced by the attenuation of UVB. Norisoprenoids are formed via the degradation of carotenoids and the higher carotenoid content in HLcontrol berries may have directly resulted in the increased levels of norisoprenoids. Additionally, the derivatives of certain carotenoids are known to perform signaling functions in plants. Ramel et al (2012) reported the rapid accumulation of β-cyclocitral upon exposure of *Arabidopsis* plants and the consequent reprogramming of gene expression

to increase the capacity for photooxidative stress tolerance. The results of that study indicated that β-cyclocitral may serve as a signaling compound in plants which leads to the activation of oxidative stress defense mechanisms. Volatile carotenoid derivatives may therefore serve as sensing and signaling compounds when plants are subjected to stress as a way to mitigate potential damage. Volatile organic compounds have been shown to increase in response to certain abiotic stresses (Possel and Loreto 2013). It is speculated that volatile terpenes (e.g. monoterpenes) play important roles in the protection of plants from environmental stress (Loreto and Schnitzler 2010; Carvalho et al., 2015). Although the exact mechanism is still unclear, the consistency of these links with stress warrants further investigation.

The higher C_6-compounds levels (e.g. n-hexanal, trans-2-hexanal) in the HLcontrol berries (versus the HL-UVB berries), indicates a role for UVB in the regulation and/or metabolism of these compounds. Leaf removal is typically used in viticulture as a canopy management strategy to reduce the "green/vegetal" character of especially red cultivars (e.g. Cabernet Sauvignon). This green character is typically associated with pyrazines (predominantly methoxypyrazines), but can also be attributed to certain C_6-compounds (e.g. hexanal) and some monoterpenes (e.g. eucalyptol) (Allen et al., 1991; Fariña et al., 2005; Lund et al., 2009). C_6-compounds are produced via the lipoxygenase-hydroperoxide lyase (LOX-HPL) pathways and are developmentally regulated and known to be released during maceration or damage. Here we show that the UVB component of light contributes to the release of C_6 compounds implicating UV in the regulation the LOX-HPL pathway and consequently the metabolism of polyunsaturated fatty acids (PUFAs). Interestingly, in the LL environment in the later developmental stages, the LLcontrol berries had significantly lower levels of the C_6-compounds relative to the LL-UVB.

Attenuation of UVB in the LL environment decreased the levels of a number of straight chain aldehydes (e.g. 2-heptanal and *trans*-2,4-heptadienal) and a ketone (1-octen-3-one). These compounds therefore reacted similarly to the C_6 compounds in the HL environment, and again implicating UVB in the metabolism of PUFAs. It is clear that the level of light exposure will determine which substrates are metabolized and/or which compounds are formed in berries, displaying considerable plasticity in these responses.

3.5.2 Control processes over non-photochemical quenching, photodamage and photorepair are activated as part of the acclimation responses and UVB plays a key role

The increase in epoxidation state of the xanthophylls (as determined by the DEPS ratio) in the HL berries is due to higher zeaxanthin levels (versus violaxanthin) in the xanthophyll pool, and is indicative of a photosynthetic system that is utilising non-photochemical quenching via zeaxanthin in the violaxanthin cycle. The response in the absence of UV (HL-UVB berries) is less than the HLcontrol,

even though the incident PAR and bunch temperature are not significantly different. UVB exposure affects the amplitude of the violaxanthin cycle response (DEPS ratio due to different zeaxanthin levels). UVB radiation is known to affect the translation of psbA (D1 protein) in the photodamage/photorepair cycle, it is likely that in the absence of UVB (as in the HL-UVB), the photosystems recover quicker (via photorepair of photodamage) than in the presence of UVB radiation (as in the HLcontrol), and/or that the actual level of saturating conditions for photosynthesis are lower in the presence of UVB radiation and HL. These results provide a hypothesis for subsequent studies on UV effects on fruit physiology and metabolism and are supported by literature from a number of fruits (Arakawa et al., 1985; Ubi et al., 2006; Calvenzani et al., 2010; Huyskens-Keil et al., 2012).

Additionally the lutein epoxide cycle is lower in the UVB attenuated LL treatments (LL-UVB). Lutein epoxide is formed in shade (deep/long term shade) and functions to protect the photosynthetic apparatus from sudden localised high light exposures (e.g. sunflecks). Although the PAR in the LLcontrol and the LL-UVB were similar (low but differing only in the incident UVB), the lutein epoxide cycle is less active in the absence of UVB (LL-UVB). It appears as if it is the UVB component of solar radiation that is required for the formation of lutein epoxide (and by extension the functioning of the lutein epoxide cycle in LL microclimate). It is evident that both cycles are required and simultaneously functional in photosynthetically active berries (albeit to varying degrees) to potentially cope with the continuously varying light conditions in the microclimate: zeaxanthin in HL and lutein epoxide in LL, with UVB affecting the absolute amounts present in photosynthetically active berries.

These responses to varying light conditions are well known and well described in photosynthetic research on photosynthetic organs (predominantly leaves); but the reports for the response of fruit to UVB exposure appears to be limited to the formation of metabolites with antioxidant or “sunscreen” activity (polyphenolics, anthocyanins, flavonols, etc.). Increased exposure of the grape berries has been shown to result in the increase of polyphenolics and certain aromatic compounds in the berry tissues (Bureau et al., 2000; Tardaguila et al., 2010; Diago et al., 2012; Gil et al., 2013; Zhang et al., 2014; Song et al., 2015). It is tempting to speculate that the formation of these latter compounds represent molecular fingerprints of long term acclimation responses of early stage (i.e. photosynthetically active) fruits attempts at protecting photosynthesis distally (by reflecting incident radiation in predominantly the exposed skins and/or via general antioxidants to mitigate the damage of reactive oxygen species). The carotenoids (specifically the xanthophylls: zeaxanthin, antheraxanthin and lutein epoxide), however, are intrinsically linked to photosynthesis and are therefore probably the more direct/local response to saturating light conditions on the photosynthetic process (as on-site antioxidants or by direct non-photochemical quenching of reactive oxygen species). It could be that it is the failure of carotenoids and other lipophilic antioxidants present in the photosynthetic membranes (of green berries), to mitigate stress that trigger the long(er) term responses involving acclimation and other photomorphogenic

responses to deal with the consequence of continued photodamage (e.g. structural changes to the skin composition and the accumulation of polyphenolics in the skin).

The metabolic outcomes of these acclimation responses and the level of stress perceived in the different microclimates clearly impacts berry composition. It has been confirmed that in both leaves (Joshi et al., 2013; Juvany et al., 2013) and berries (Carbonell-Bejerano et al., 2014; Lui et al., 2015) young photosynthetically active tissues respond differently to increased exposure compared to older tissue (old, senescing leaves or ripe berries). Figure 3.7 proposes an overview model of the respective responses and highlights the importance of the developmental stage (early or late) as well as the microclimate (HL or LL) on the metabolites that are differentially produced and proposed to play a role in the acclimation responses. The data presented supports the hypothesis that plants in shade are less acclimated and consequently more susceptible (on e.g. a clear day) than the exposed (HL) more acclimated counterparts (typically displaying higher flavonols, higher photo-protective xanthophylls, and/or antioxidant volatiles, depending on the developmental stage). In the absence of UVB, less acclimation has potentially occurred in the LL-UVB and the plants will be more susceptible (to e.g. sunflecks) than the more acclimated HL-UVB counterparts. Here we show that these general plant responses are active in grapevine berries with developmental stages displaying distinctive responses.

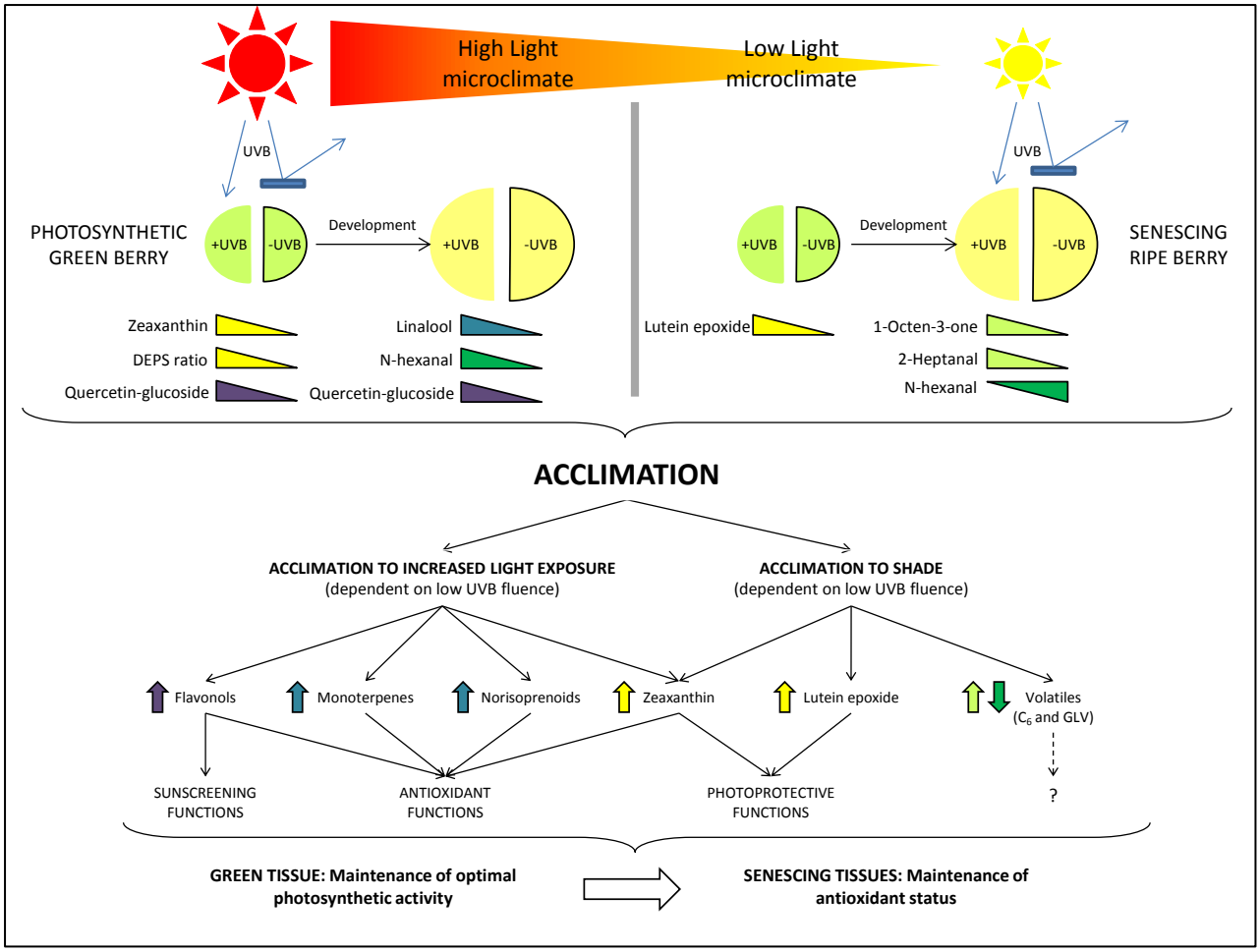


Figure 3.7. A comprehensive model summarizing the results of the study**.** In each light environment (HL and LL) both early and late developmental stages are represented as well as the attenuation of UVB. The coloured triangles indicate those compounds which reacted to UVB attenuation in each case, indicating the presence of an acclimation response in the berries. Each of the compound groups perform a specific function in the berry tissue and contribute to the acclimation of the berry via various physiological processes. These processes differ depending on the tissue type and are therefore associated with the developmental stage of the berry.

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Author contribution

MAV conceived and planned the study. CJ implemented and maintained the viticultural treatments and monitored the vineyard. CJ and PY carried out berry sampling. CJ did the climatic data processing and analysis, processing and analysis of the berry samples together with HE which performed the UPLC, HPLC and GC-MS analysis. CJ performed the data integration and processing for the above compounds. CJ, HE and PY performed data analysis. CJ, PY and MV drafted the initial manuscript, all authors contributed to the final manuscript.

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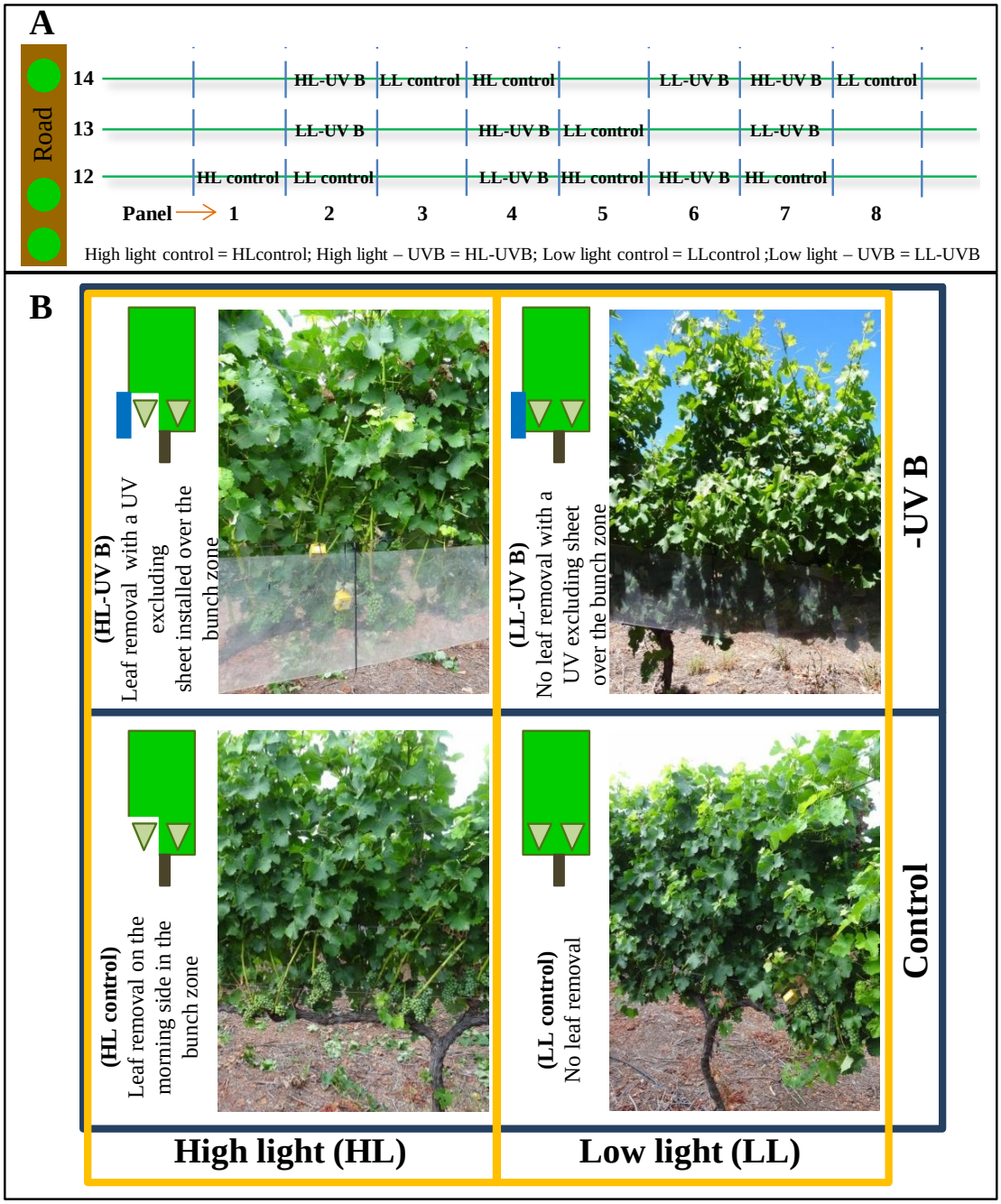
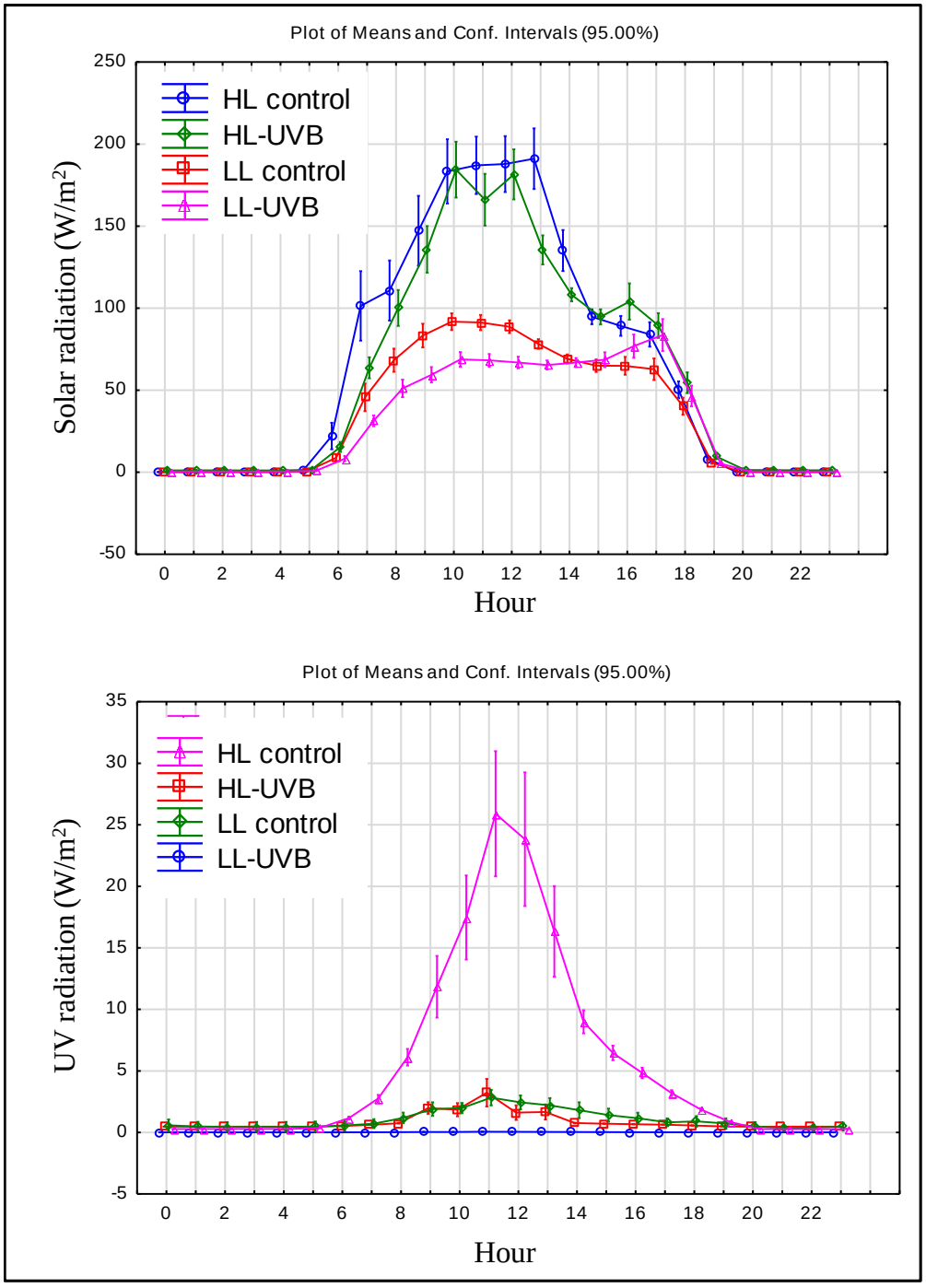
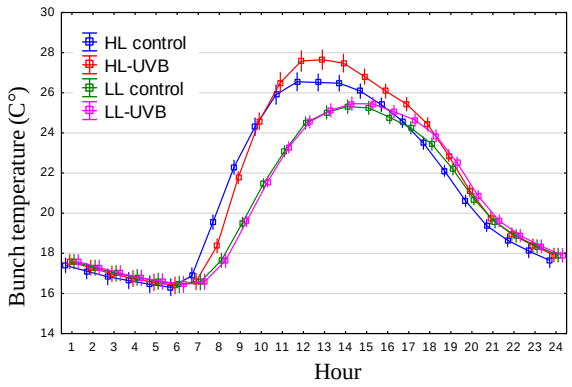
Supplementary data to Chapter 3

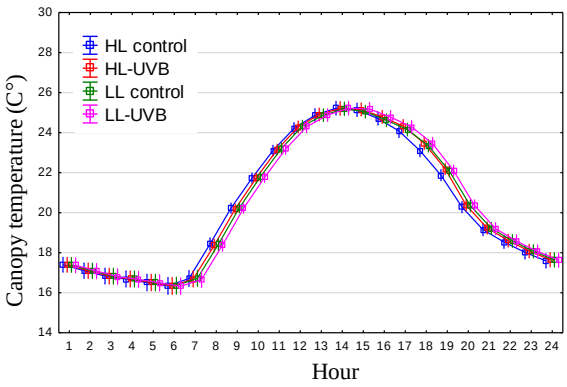


Supplementary figure 3.1. The experimental layout of the treatments within the plot **A)** and the four light environments created by leaf removal and UVB attenuation **B).**



Supplementary figure 3.2. The mean hourly seasonal from berry set to harvest) solar radiation and UV radiation data mean ± 95% confidence interval) for each light environment measured in the 2014/2015 experimental season. The first hour is from 00h00 to 01h00.

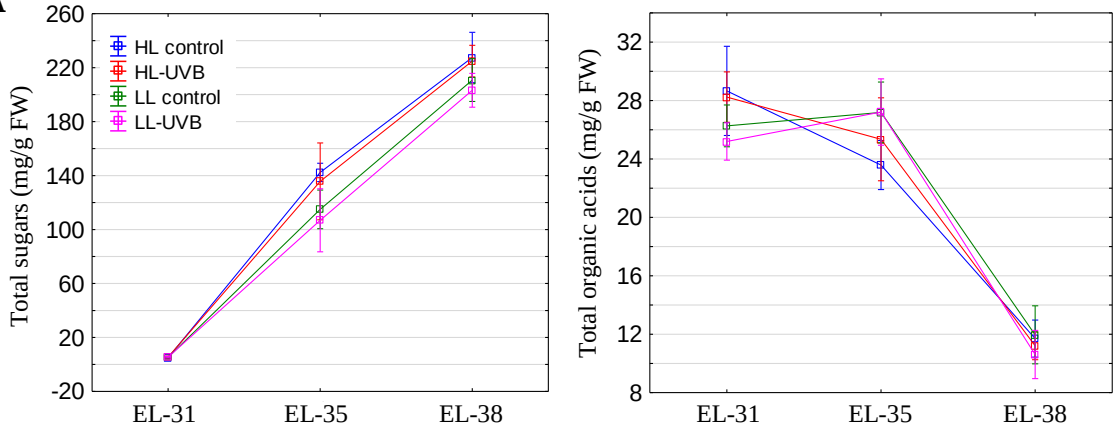




Parameters		High light		Low light	
		+ UV	- UV B	+ UV	- UV B
Bunch temperature (C°)	Mean±SD	21.1±5.3	21.4±5.6	20.4±4.6	20.5±4.7
	Minimum	7.4	7.8	8	8
	Maximum	37.1	39.6	33.7	33.8
Canopy temperature (C°)	Mean±SD	20.3±4.6	20.4±4.7	20.4±4.6	20.4±4.7
	Minimum	7.4	7.4	7.4	7.3
	Maximum	33.9	34.1	34	34.1

Supplementary figure 3.3. The seasonal 2014/2015) bunch and canopy minimum, maximum and mean ± SD) temperatures for all light environments and the corresponding kinetics showing the mean hourly bunch and canopy temperatures mean ± 95% confidence interval) measured in the 2014/2015 experimental season. The first hour is from 00h00 to 01h00.

A

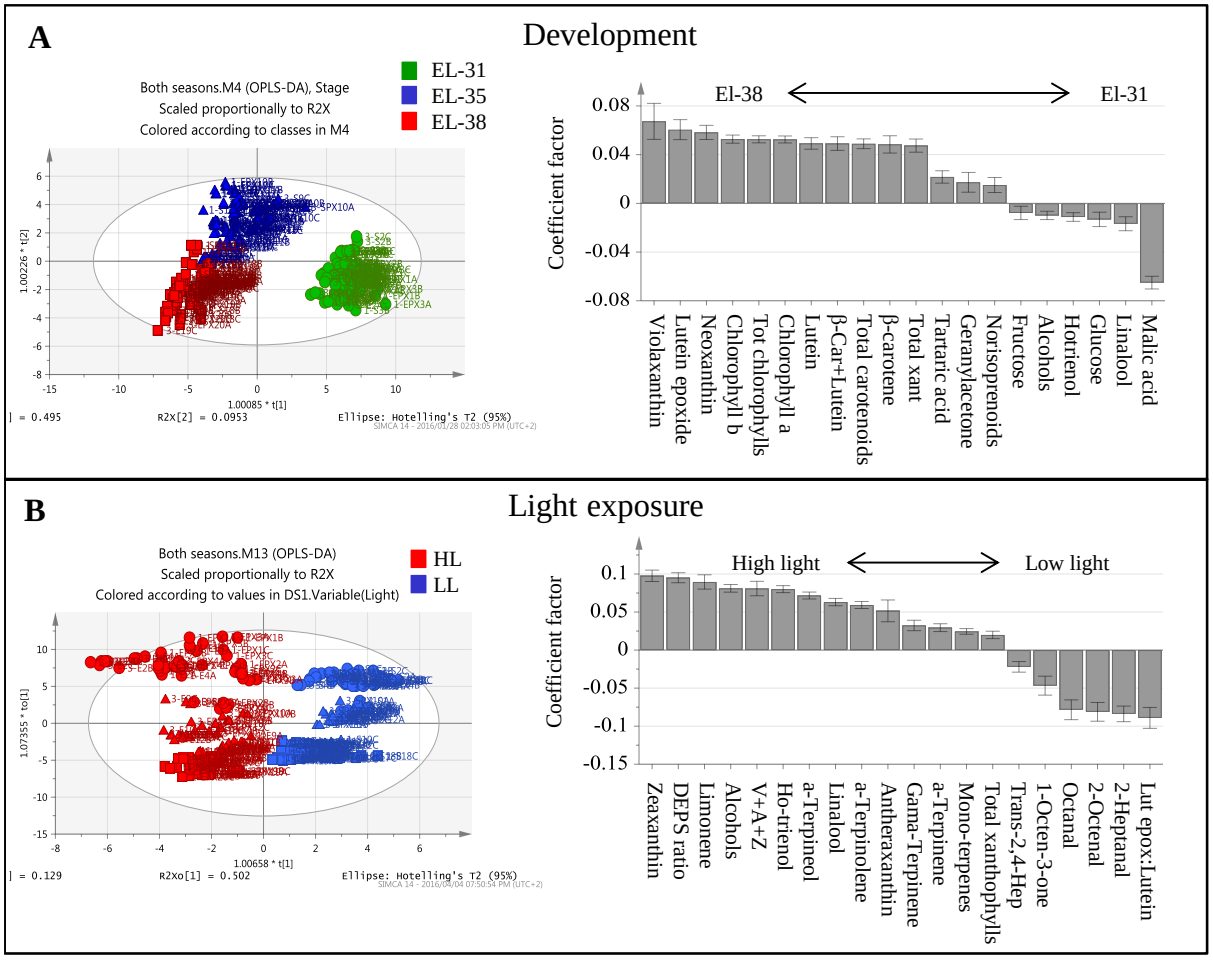


Ripening	High light		Low light	
Parameters	Control	- UV B	Control	- UV B
Brix	24.4	25.1	23.9	24.2
pH	3.39	3.31	3.31	3.29
TA	6.53	6.77	7.38	7.31

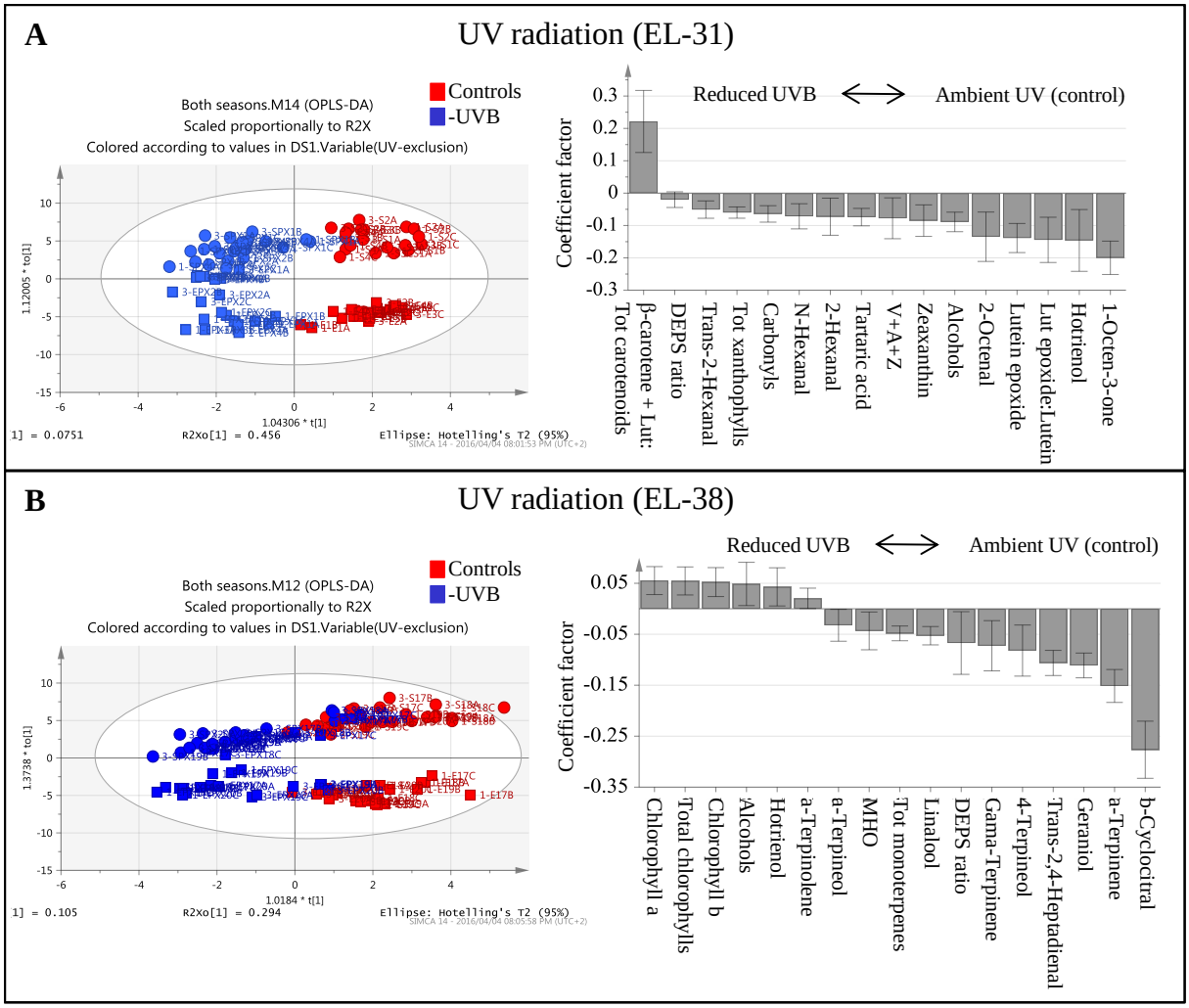
B

Ripening	High light		Low light	
Parameters	Control	- UV B	Control	- UV B
Brix	22.23±0.01	22.23±0.01	21.13±0.01	21.2±0
pH	3.36±0	3.3±0	3.01±0	2.98±0.01
TA	11.57±0	11.53±0.01	12.66±0.01	12.78±0.01

Supplementary figure 3.4. A. The total sugars and total organic acid contents measured over berry development and the ripening parameters determined at harvest (2011/2012 season). **B**. The ripening parameters measured for the last experimental season (2014/2015 season).



Supplementary figure 3.5. OPLS-DA models generated for all metabolic data over both experimental seasons for developmental stage **A)** and light exposure **B)**. Each OPLS-DA is accompanied by a co-efficient plot of compounds which contributed most to the respective models. These were chosen according to the individual variable importance plots VIP's) and included the top compounds with a VIP ≥0.5. Shapes of the sample icons denote the respective developmental stages: EL-31 ●), EL-35 ▲) and EL-38 ■).



Supplementary figure 3.6. OPLS-DA models generated for all metabolic data over both experimental seasons for the early A) and late B) developmental stages separately. The attenuation of UVB was used as the y-factor in both models. Each OPLS-DA is accompanied by a co-efficient plot of compounds which contributed most to the respective models. These were chosen according to the individual variable importance plots VIP's) and included the top compounds with a value above 0.5. Shapes of the sample icons denote the respective exposure: High Light ■) and Low Light ●).

Supplementary table 3.1. Calibration curve of volatile organic compounds used in this study and analysed by HS-SPME and GC single-quadrupole-MS.

Compounds	Ion	Quantification range µg/L)	y-intercept	Slope	LOD µg/L)	LOQ µg/L)	r^2
β-Damascone	177	0-8.8	-0.005	0.026	0.67	2.05	0.9965
α-ionone	177	0-8.9	0.000	0.001	0.51	1.55	0.9975
β-ionone	177	0-7.8	-0.004	0.018	0.94	2.86	0.9916
Limonene	93	0.18.9	0.002	0.001	6.83	20.69	0.9225
Linalool	93	0-18.9	0.001	0.019	1.60	4.84	0.9960
α-Terpineol	93	0-4.7	-0.001	0.044	0.25	0.76	0.9986
Linalool-Oxide	111	0-8.2	0.000	0.001	0.92	2.78	0.9927
Trans-2-Hexanal	83	0-95.7	0.005	0.002	20.02	60.68	0.9708
1-Hexanol	55	0-10.5	-0.001	0.015	0.37	1.11	0.9993
2-Heptenal	55	0-40	0.005	0.010	4.83	14.63	0.9901
MHO	108	0-7.5	-0.001	0.016	0.35	1.06	0.9987
2-Octanal	55	0-24.6	0.000	0.020	1.05	3.17	0.9988
4-Terpineol	71	0-10	-0.008	0.076	0.31	0.93	0.9995
Citronellol	93	0-7	-0.003	0.024	0.45	1.36	0.9976
Nerol	93	0-8.6	0.000	0.013	0.52	1.58	0.9979
β-damascenone	93	0-8	-0.017	0.121	0.42	1.28	0.9984
Geraniol	93	0-6	-0.003	0.051	0.29	0.89	0.9986
Geranylactone	93	0-34.4	0.005	0.023	0.89	2.99	0.9994
Pseudo-ionone	124	0-8.3	0.000	0.001	1.00	2.17	0.9958

Supplementary table 3.2. Selected ions used for the integration of the peak area of the respective compounds of interest as well as their retention time on the Zebron column and quantifier molecules analysed by HS-SPME and GC single-quadrupole-MS.

Compounds	RT min)	Ion	Quantifier
N-Hexanal	4.76	82	Trans-2-Hexanal
β-Myrcene	4.94	93	Limonene
α-Pinene	5.78	93	Limonene
α-terpinene	5.96	93	Limonene
Limonene	6.26	93	Limonene
Sabinene	6.41	93	Limonene
Cineol	6.49	93	Limonene
2-Hexanal	6.56	83	Trans-2-Hexanal
Trans-2-Hexanal	6.86	83	Trans-2-Hexanal
Gama-Terpinene	7.03	93	Limonene
α-Terpinolene	7.62	93	Limonene
Octanal	7.86	55	2-Octenal
1-Octen-3-one	8.05	55	MHO
2-Heptanal	8.41	55	2-Heptanal
MHO	8.58	108	MHO
ISAnisol)	8.67	116	-
1-Hexanol	8.77	55	1-Hexanol
3-Hexanol	9.20	55	1-Hexanol
Nonenal	9.34	55	2-Octenal
Fenchone	9.32	81	Fenchone

2,4-Hexadienal	9.51	81	Trans-2-Hexanal
2-Octenal	9.86	55	2-Octenal
Cis-Linolool oxide	9.97	111	Cis-Linolool oxide
Trans-Linalool oxide	10.35	111	Trans-Linalool oxide
Cis-2,4-Heptadienal	10.38	81	2-Heptanal
Trans-2,4-Heptadienal	10.77	81	2-Heptanal
2-Nonenal	11.23	93	2-Octenal
Linalool	11.29	93	Linalool
Junipene	11.57	93	Linalool
Trans-b-caryophyllene	11.73	93	Linalool
Trans-b-caryophyllene	11.92	93	Linalool
4-Terpineol	12.03	71	4-Terpineol
Hotrienol	12.09	71	1-Hexanol
b-Cyclocitral	12.29	123	Linalool
2-Decanal	12.50	93	2-Octenal
α-Humulene	12.81	93	Linalool
α-Humulene	12.87	93	Linalool
a-Terpineol	13.14	93	a-Terpineol
Trans-trans-nona-2,4-dienal	13.31	81	2-Octenal
α-Farnesene	13.54	93	a-Terpineol
EE-α-Farnesene	13.77	93	a-Terpineol
Delta-Cadinene	13.87	161	Linalool
2,4-Nonadienal	13.99	81	2-Octenal
Citronellol	13.83	69	Citronellol
Nerol	14.31	69	Nerol
2,4-Nonadienal	14.51	81	2-Octenal
β-damascone	14.53	177	β-damascone
β-Damascenone	14.58	69	β-Damascenone
Geraniol	14.81	69	Geraniol
Geranylacetone	14.91	69	Geranylacetone
α-ionone	14.91	177	α-ionone
Propanoic acid	15.22	69	2-Octenal
Cis-Farnesol	15.69	69	1-Hexanol
β-ionone	15.83	177	β-ionone
Trans-β-ionone-5,6-epoxide	16.40	123	β-ionone
Trans-β-ionone-5,6-epoxide	16.54	123	β-ionone
Pseudo-ionone	16.87	124	Pseudo-ionone
Pseudo-ionone	17.76	124	Pseudo-ionone
Nonanoic acid	18.01	124	2-Octenal

Supplementary table 3.3. An analysis of the metabolic data from the first experimental season 2011/2012 season). The repeated measures ANOVA results for the listed parameters and individual compounds are reported as F-values. Values are scaled from highest most significant) to lowest by colour. Green indicates low F-values significant), while red indicates high F-values values more significant). All insignificant values $F \leq 3$) are colored in gray. Maximum ■; 50% ■; minimum ■; insignificant ■

	Development	Exposure	UVB-attenuation	Exposure × Development	UVB-attenuation × Development	UVB-attenuation × Exposure	UVB-attenuation × Exposure × Development
Total chlorophylls	1205.26	0.00	0.13	22.05	4.69	0.49	8.25
Glucose	1126.48	7.49	0.46	4.82	0.30	0.07	0.05
Total sugars	1060.99	8.53	0.57	4.93	0.37	0.04	0.03
Fructose	1000.92	9.63	0.69	5.02	0.45	0.02	0.02
Total carotenoids	859.01	6.88	5.77	41.33	13.06	0.59	14.15
Total xanthophylls	562.22	39.86	19.80	38.81	40.66	4.12	18.56
Total acids	395.67	0.00	0.32	9.30	1.36	1.25	0.09
Tartaric acid	329.09	15.00	0.17	1.84	0.76	0.15	0.16
Malic acid	206.64	35.33	0.01	11.92	1.45	1.82	0.48
Succinic acid	125.14	14.57	0.29	10.82	0.04	0.01	0.00

Supplementary table 3.4. A table listing the measured contents of all the compounds ± SD for both experimental seasons. The log_2-fold changes and corresponding p-values between the HL control/ HL-UVB and LL control)/LL-UVB contrasts are calculated and listed for each compound at each developmental stage.

Compound	EL31: HLcontrol	EL31: HL-UVB	Fold change (log2)	p-value	EL31: LLcontrol	EL31: LL-UVB	Fold change (log2)	p-value
Sugars and organic acids (2011/2012)								
Citric acid	0.19±1.65	0.18±0.35	0.08	0.14	0.13±0.6	0.12±0.78	0.12	0.04
Tartaric acid	15.77±0.08	15.49±0.08	0.03	0.00	14.53±0.05	14.16±0.05	0.04	0.61
Malic acid	6.06±0.18	5.96±0.1	0.02	0.00	7.1±0.05	6.44±0.05	0.14	0.03
Succinic acid	6.65±0.21	6.61±0.08	0.01	0.00	4.51±0.15	4.49±0.15	0.01	0.87
Glucose	3.98±0.18	4.17±0.16	-0.07	0.00	4.16±0.05	4.21±0.11	-0.02	0.54
Fructose	0.98±0.19	1.3±0.09	-0.41	0.00	1.08±0.09	1.24±0.1	-0.20	0.35
Photosynthetic pigments (2011/2012)								
Neoxanthin	2913.85±0.15	2050.67±0.11	0.51	0.00	2406.76±0.26	2121.64±0.21	0.18	0.30
Violaxanthin	1902.5±0.19	1790.42±0.13	0.09	0.00	2001.21±0.26	1573.26±0.21	0.35	0.73
Lutein epoxide	1155.46±0.15	813.81±0.44	0.51	0.00	2049.47±0.03	1199.36±0.19	0.77	0.87
Antheraxanthin	925.56±0.14	552.17±0.11	0.75	0.00	119.28±0.19	121.26±0.21	-0.02	0.24
Lutein	9716.46±0.11	6286.62±0.12	0.63	0.00	5512.4±0.09	5131.84±0.2	0.10	0.02
Zeaxanthin	5024.69±0.09	1588.25±0.29	1.66	0.00	296.5±0.06	124.82±1.49	1.25	0.64
Chlorophyll b	38230.48±0.13	33528.22±0.09	0.19	0.00	31840.78±0.04	31955.65±0.05	-0.01	0.31
Chlorophyll a	88648.24±0.12	74035.63±0.16	0.26	0.00	68088.42±0.08	71640.89±0.07	-0.07	0.18
β-carotene	39802.16±0.14	32769.05±0.12	0.28	0.00	28280.79±0.07	28116.6±0.04	0.01	0.00
Total chlorophylls	126878.72±0.13	107563.86±0.14	0.24	0.00	88133.99±0.35	103596.54±0.05	-0.23	0.22
Total carotenoids	61440.68±0.12	45851±0.11	0.42	0.00	30189.21±0.52	38388.77±0.06	-0.35	0.00
Total xanthophylls	21638.51±0.09	13081.95±0.09	0.73	0.00	11149.61±0.38	10272.17±0.2	0.12	0.43
Chlorophyll a:Chlorophyll b (ratio)	2.32±0.02	2.19±0.1	0.08	0.05	1.8±0.47	2.25±0.08	-0.32	0.16
Carotene:Chlorophyll (ratio)	0.31±0.02	0.31±0.03	0	0.45	0.19±0.74	0.27±0.06	-0.51	0.02
β-Carotene+Lutein	49518.62±0.13	39055.67±0.12	0.34	0.00	24256.01±0.59	33248.44±0.05	-0.45	0.00
β-carotene+Lutein:Total Carotenoids (ratio)	0.81±0.01	0.85±0.01	-0.07	0.00	0.71±0.41	0.87±0.03	-0.29	0.00
V+A+Z	7852.75±0.07	3930.85±0.18	1	0.00	2178.88±0.58	1819.34±0.23	0.26	0.65
DEPS (ratio)	0.76±0.05	0.54±0.11	0.49	0.00	0.17±0.5	0.13±0.53	0.39	0.55
Lx:L (ratio)	0.12±0.11	0.14±0.7	-0.22	0.00	0.31±0.48	0.24±0.08	0.37	0.15
Photosynthetic pigments (2014/2015)								
Neoxanthin	3071.24±0.05	3160.7±0.05	-0.04	0.20	2885.26±0.09	2590.8±0.05	0.16	0.00
Violaxanthin	1435.12±0.04	1372.79±0.11	0.06	0.18	1228.75±0.04	1230.93±0.05	0.00	0.92
Lutein epoxide	1302.51±0.09	1513.86±0.17	-0.22	0.01	2764.29±0.04	2004.66±0.1	0.46	0.00
Antheraxanthin	821.26±0.11	698.64±0.11	0.23	0.00	137.74±0.13	216.74±0.28	-0.65	0.00
Lutein	10327.98±0.06	11041.39±0.04	-0.10	0.00	7789.39±0.09	7948.8±0.1	-0.03	0.59
Zeaxanthin	4747.6±0.19	2681.97±0.28	0.82	0.00	597.22±0.17	351.95±0.11	0.76	0.00
Chlorophyll b	36179.5±0.06	40101.67±0.04	-0.15	0.00	32922.67±0.05	34461.6±0.07	-0.07	0.08
Chlorophyll a	86623.92±0.09	92989.4±0.05	-0.10	0.02	74231.38±0.07	76915.82±0.11	-0.05	0.36
β-carotene	36825.61±0.06	41292.02±0.05	-0.17	0.00	31069.99±0.09	31160.72±0.08	0.00	0.94
Total chlorophylls	122803.42±0.08	133091.07±0.04	-0.12	0.01	107154.04±0.06	111377.42±0.1	-0.06	0.26
Total carotenoids	58531.32±0.04	61761.37±0.04	-0.08	0.00	46472.63±0.06	45504.6±0.07	0.03	0.44
Total xanthophylls	21705.71±0.04	20469.35±0.04	0.08	0.00	15402.64±0.07	14343.88±0.07	0.10	0.02
Chlorophyll a:Chlorophyll b (ratio)	2.39±0.03	2.32±0.03	0.04	0.04	2.25±0.02	2.23±0.06	0.02	0.58
Carotene:Chlorophyll (ratio)	0.3±0.05	0.31±0.03	-0.04	0.09	0.29±0.13	0.28±0.03	0.06	0.32
β-Carotene+Lutein	47153.6±0.06	52333.4±0.04	-0.15	0.00	38859.38±0.07	39109.52±0.08	-0.01	0.83
β-carotene+Lutein:Total Carotenoids (ratio)	0.81±0.02	0.85±0.01	-0.08	0.00	0.84±0.01	0.86±0.01	-0.04	0.00
V+A+Z	7003.98±0.14	4753.41±0.16	0.56	0.00	1963.71±0.07	1799.62±0.07	0.13	0.01
DEPS (ratio)	0.79±0.04	0.7±0.1	0.18	0.00	0.37±0.1	0.31±0.09	0.24	0.00
Lx:L (ratio)	0.13±0.08	0.14±0.14	-0.15	0.11	0.36±0.1	0.25±0.16	0.49	0.00

Volatile compounds (2014/2015)								
β-ionone	10.52±0.34	14.93±0.42	-0.51	0.04	14.87±0.28	16.56±0.38	0.00	0.45
β-Cyclocitral	2.38±0.19	2.97±0.32	-0.32	0.06	2.84±0.12	3.27±0.27	0.00	0.13
β-Damascenone-69								0.00
Geranylacetone	6.36±0.36	5.87±0.23	0.11	0.53	21±0.41	30.5±0.15	0.06	0.00
MHO	1.49±0.38	1.15±0.66	0.38	0.21	5.16±0.47	8.25±0.22	-0.68	0.00
Limonene	40.21±0.27	47.84±0.37	-0.25	0.22	30.25±0.3	25.82±0.69	0.00	0.45
α-Terpinene	191.24±0.23	216.68±0.38	-0.18	0.36	116.49±0.25	87.24±0.21	0.00	0.01
Cineol	400.18±0.34	345.86±0.31	0.21	0.29	380.18±0.26	283.35±0.12	0.00	0.00
Gamα-Terpinene	318.67±0.21	397.53±0.33	-0.32	0.08	191.66±0.28	221.27±0.24	0.00	0.19
α-Terpinolene	123.17±0.19	132.92±0.23	-0.11	0.38	59.29±0.26	52.78±0.21	0.00	0.25
Linalool	3.11±0.63	3.74±0.1	-0.27	0.28	2.1±0.89	1.94±0.88	-0.37	0.83
α-Terpineol	6.21±0.24	6.34±0.15	-0.03	0.80	3.15±0.2	2.78±0.27	0.00	0.22
Geraniol	1.39±0.38	1.51±0.39	-0.12	0.61	1.37±0.34	0.31±1.05	0.00	0.00
4-Terpineol	5.06±0.23	5.24±0.15	-0.05	0.66	3.26±0.1	2.88±0.11	0.00	0.01
Sabinene	30.97±0.3	34.29±0.55	-0.15	0.59	19.41±0.41	14.39±0.49	0.43	0.12
Trans-2-Hexanal	2986.45±0.26	3670.59±0.37	-0.30	0.14	3365.92±0.17	2613.62±0.38	0.36	0.03
2-Hexanal	65.54±0.27	76.48±0.51	-0.22	0.39	70.7±0.24	34.77±0.9	1.02	0.00
N-Hexanal	207.66±0.48	407.58±0.7	-0.97	0.03	404.14±0.54	96.59±0.89	2.06	0.00
1-Octen-3-one	4.73±0.7	4.55±0.5	0.06	0.88	42.38±0.37	6.29±1.15	2.75	0.00
2-Heptanal	22.35±0.17	18.28±0.49	0.29	0.16	80.17±0.24	71.46±0.16	0.17	0.19
Trans-2,4-Heptadienal	26.86±0.26	36.29±0.44	-0.43	0.08	50.77±0.15	38.81±0.12	0.39	0.00
2-Octenal	15.89±0.22	10.29±0.86	0.63	0.05	48.44±0.22	36.2±0.15	0.42	0.00
Octanal		1.2±1.79		0.07	3.49±0.19	5.16±0.5	-0.56	0.04
Nonenal	3.01±0.72	7.59±0.75	-1.33	0.02	5.05±0.4	4.42±0.49	0.19	0.46
3-Hexanol	1.35±1.34	6.55±0.63	-2.28	0.00	4.06±0.74		1.76	0.00
Hotrienol	21±0.19	14.24±0.25	0.56	0.00	8.74±0.23	30.55±0.2	0.65	0.00
Norisoprenoids	20.75±0.29	24.93±0.33	-0.26	0.16	43.88±0.23	58.58±0.12	-0.07	0.00
Mono-terpenes	1089.24±0.23	1157.66±0.23	-0.09	0.52	787.75±0.14	678.37±0.17	0.00	0.03
Carbonyl compounds	3363.48±0.26	4267.14±0.39	-0.34	0.11	4090.46±0.19	2921.71±0.38	0.49	0.01
Alcohols	22.35±0.18	20.79±0.28	0.10	0.46	12.79±0.31	30.55±0.2	0.92	0.00
C6 compounds	3261.01±0.27	4161.19±0.39	-0.35	0.11	3844.81±0.2	2744.98±0.4	0.66	0.01
Compound	**EL35: HLcontrol**	**EL35: HL-UVB**	**Fold change (log2)**	**p-value**	**EL35: LLcontrol**	**EL35: LL-UVB**	**Fold change (log2)**	**p-value**
Sugars and organic acids (2011/2012)								
Citric acid	0.35±0.24	0.29±0.14	0.27	0.00	0.33±0.34	0.31±0.11	0.09	0.56
Tartaric acid	10.83±0.13	11.09±0.09	-0.03	0.00	10.37±0.07	10.68±0.09	-0.04	0.97
Malic acid	7.1±0.11	8.73±0.27	-0.3	0.00	12.15±0.12	12.03±0.14	0.01	0.18
Succinic acid	5.31±0.19	5.24±0.2	0.02	0.00	4.34±0.17	4.19±0.17	0.05	0.27
Glucose	74.9±0.05	72.28±0.2	0.05	0.00	62.08±0.12	58.34±0.2	0.09	0.77
Fructose	67.45±0.05	63.41±0.23	0.09	0.00	52.87±0.13	48.53±0.25	0.12	0.59
Photosynthetic pigments (2011/2012)								
Neoxanthin	598.67±0.5	631.44±0.33	-0.08	0.00	947.89±0.09	891.27±0.45	0.09	0.00
Violaxanthin	75.87±1.82	192.49±0.23	-1.34	0.00	674.6±0.11	708.28±0.53	-0.07	0.00
Lutein epoxide	42.67±1.85	269.01±0.24	-2.66	0.00	638.22±0.09	377.75±0.31	0.76	0.00
Antheraxanthin	130.36±0.59	181.92±0.24	-0.48	0.30	126.28±0.1	149.85±0.3	-0.25	0.57
Lutein	3267.27±0.29	2833.64±0.09	0.21	0.00	2959.39±0.05	3050.63±0.18	-0.04	0.07
Zeaxanthin	4280.14±0.12	3773.59±0.06	0.18	0.00	858.2±0.11	821.73±0.19	0.06	0.01
Chlorophyll b	11641.35±0.41	12326.84±0.22	-0.08	0.00	14692.3±0.06	15610.11±0.13	-0.09	0.01
Chlorophyll a	27867.09±0.34	27390.11±0.19	0.02	0.00	32732.13±0.04	34813.45±0.1	-0.09	0.01
β-carotene	11510.29±0.46	9904.98±0.18	0.22	0.00	14662.95±0.05	14174.76±0.08	0.05	0.00
Total chlorophylls	39508.45±0.36	39716.96±0.2	-0.01	0.00	47424.43±0.05	50423.56±0.1	-0.09	0.01
Total carotenoids	19905.27±0.36	17787.07±0.12	0.16	0.00	20867.54±0.04	20174.28±0.08	0.05	0.01
Total xanthophylls	8394.99±0.24	7882.09±0.07	0.09	0.00	6204.58±0.04	5999.52±0.23	0.05	0.72
Chlorophyll a:Chlorophyll b (ratio)	2.44±0.05	2.24±0.04	0.12	0.00	2.23±0.03	2.24±0.04	-0.01	0.06
Carotene:Chlorophyll (ratio)	0.29±0.3	0.25±0.07	0.21	0.02	0.31±0.04	0.28±0.1	0.15	0.83
β-Carotene+Lutein	14777.56±0.42	12738.63±0.15	0.21	0.00	17622.34±0.04	17225.39±0.07	0.03	0.00
β-carotene+Lutein:Total Carotenoids (ratio)	0.73±0.07	0.71±0.03	0.04	0.00	0.84±0.01	0.86±0.04	-0.03	0.00

V+A+Z	4486.37±0.15	4147.99±0.06	0.11	0.00	1659.09±0.04	1679.86±0.25	-0.02	0.08
DEPS (ratio)	0.99±0.03	0.95±0.01	0.06	0.00	0.59±0.07	0.6±0.16	-0.02	0.00
Lx:L (ratio)	0.01±1.83	0.1±0.28	-3.32	0.00	0.22±0.07	0.12±0.3	0.87	0.00
Photosynthetic pigments (2014/2015)								
Neoxanthin	279.05±0.16	241.67±0.2	0.21	0.06	357.68±0.17	312.41±0.11	0.20	0.03
Violaxanthin	273.03±0.19	179.25±0.3	0.61	0.00	459.82±0.16	359.19±0.12	0.36	0.00
Lutein epoxide	147.99±0.13			0.00	254.64±0.22	177.89±0.18	0.52	0.00
Antheraxanthin	407.74±0.11	271.28±0.24	0.59	0.00	218.98±0.23	221.01±0.18	-0.01	0.91
Lutein	2745.84±0.19	2516.41±0.14	0.13	0.22	3127.74±0.07	2761.24±0.08	0.18	0.00
Zeaxanthin	2002.93±0.15	1512.13±0.18	0.41	0.00	603.16±0.14	519.46±0.11	0.22	0.01
Chlorophyll b	3220.66±0.23	3107.31±0.18	0.05	0.68	4125.69±0.09	3671.43±0.1	0.17	0.01
Chlorophyll a	6820.68±0.18	6425.94±0.14	0.09	0.37	8271.29±0.06	7358.6±0.08	0.17	0.00
β-carotene	1028.72±0.19	925.07±0.14	0.15	0.14	1318.46±0.1	1150.4±0.09	0.20	0.00
Total chlorophylls	10041.33±0.19	9533.25±0.15	0.07	0.48	12396.97±0.07	11030.03±0.09	0.17	0.00
Total carotenoids	6885.29±0.1	5645.81±0.06	0.29	0.00	6340.49±0.05	5501.6±0.07	0.20	0.00
Total xanthophylls	5856.57±0.08	4720.74±0.05	0.31	0.00	5022.03±0.05	4351.2±0.07	0.21	0.00
Chlorophyll a:Chlorophyll b (ratio)	2.14±0.05	2.08±0.04	0.04	0.17	2.01±0.03	2.01±0.03	0.00	0.97
Carotene:Chlorophyll (ratio)	0.1±0.07	0.1±0.08	0.00	0.12	0.11±0.07	0.1±0.06	0.03	0.48
β-Carotene+Lutein	3774.55±0.19	3441.48±0.14	0.13	0.19	4446.2±0.07	3911.64±0.08	0.18	0.00
β-carotene+Lutein:Total Carotenoids (ratio)	0.54±0.1	0.61±0.08	-0.18	0.01	0.7±0.04	0.71±0.02	-0.02	0.32
V+A+Z	2683.7±0.1	1962.66±0.1	0.45	0.00	1281.96±0.12	1099.66±0.08	0.22	0.00
DEPS (ratio)	0.9±0.03	0.91±0.03	-0.02	0.37	0.64±0.09	0.67±0.05	-0.07	0.09
Lx:L (ratio)	0.06±0.18			0.00	0.08±0.29	0.06±0.19	0.36	0.03
Volatile compounds (2014/2015)								
β-ionone	11.32±0.2	9.68±0.19	0.23	0.07	9.97±0.16	10.27±0.17	0.00	0.66
β-Cyclocitral	2.46±0.11	1.93±0.34	0.35	0.02	2.43±0.13	2.35±0.15	0.00	0.55
β-Damascenone-69	3.51±0.08	3.68±0.15	-0.07	0.35	2.82±0.12	3.26±0.13	0.00	0.01
Geranylacetone	22.11±0.3	10.18±0.63	1.12	0.00	31.64±0.38	37.39±0.22	0.00	0.19
MHO	5.43±0.39	1.8±0.81	1.59	0.00	8.35±0.29	9.28±0.19	-0.15	0.30
Limonene	18.04±0.21	17.35±0.18	0.06	0.63				0.00
α-Terpinene	30.71±0.51	48.01±0.39	-0.64	0.02	16.5±0.5	17.23±0.39	0.01	0.81
Cineol					1.19±3.46	1.42±3.46		0.91
Gamα-Terpinene	66.65±0.36	85.44±0.47	-0.36	0.18	50.36±0.25	40.81±0.25	0.04	0.06
α-Terpinolene	36.15±0.24	35.24±0.35	0.04	0.84	19.09±0.53	12.15±0.92	0.04	0.12
Linalool	15.08±0.48	9.55±0.57	0.66	0.05	2.16±1.12	1.45±1.24	0.00	0.42
α-Terpineol	2.51±0.1	1.2±1.09	1.06	0.00	1.37±0.22	1.05±0.25	0.00	0.01
Geraniol	1.24±0.2	0.86±0.18	0.53	0.00	1.02±0.29	1.11±0.29	0.00	0.45
4-Terpineol	1.07±0.18	1.03±0.26	0.05	0.68	0.75±0.34	0.61±0.5	0.01	0.22
Sabinene	1.58±3.46			0.33				0.00
Trans-2-Hexanal	6150.54±0.31	6336.05±0.31	-0.04	0.82	8079.89±1.62	3265.43±0.16	1.31	0.22
2-Hexanal	144.39±0.44	41.41±1.83	1.80	0.00	159.53±1.3	90.74±0.16	0.81	0.26
N-Hexanal	884.06±0.21	631.13±0.3	0.49	0.00	249.6±0.58	445.73±0.18	-0.84	0.00
1-Octen-3-one	18.98±0.26	8.92±0.48	1.09	0.00	30.84±0.68	53.23±0.16	-0.79	0.00
2-Heptanal	27.76±0.22	14.4±0.32	0.95	0.00	50.8±0.37	61.64±0.23	-0.28	0.12
Trans-2,4-Heptadienal	19.09±0.22	22.02±0.2	-0.21	0.11	22.8±0.29	23.89±0.33	-0.07	0.72
2-Octenal	19.26±0.22	11.71±0.41	0.72	0.00	28.47±0.37	37.94±0.15	-0.41	0.01
Octanal	2.8±0.14	0.6±1.82	2.22	0.00	1.42±1.06	3.6±0.15	-1.34	0.00
Nonenal	6.83±0.09	4.38±0.96	0.64	0.06	4.38±0.29	7.9±0.18	-0.85	0.00
3-Hexanol	0.47±1.82	2.32±1.13	-2.30	0.03	0.58±1.14		0.47	0.01
Hotrienol	47.82±0.37	29.37±0.37	0.70	0.01	12.78±0.43	6.61±0.18	0.83	0.00
Norisoprenoids	44.84±0.18	27.26±0.35	0.72	0.00	55.21±0.28	62.55±0.18	-0.02	0.20
Mono-terpenes	171.45±0.25	198.68±0.32	-0.21	0.23	92.44±0.31	75.84±0.38	0.04	0.17
Carbonyl compounds	7275.29±0.29	7070.61±0.31	0.04	0.82	8627.74±1.54	3990.11±0.15	1.11	0.24
Alcohols	48.3±0.37	31.69±0.4	0.61	0.02	13.36±0.43	6.61±0.18	0.81	0.00
C6 compounds	7179.46±0.29	7010.91±0.31	0.03	0.85	8489.61±1.57	3801.91±0.16	1.16	0.24
Compound	**EL38: HLcontrol**	**EL38: HL-UVB**	**Fold change (log2)**	**p-value**	**EL38: LLcontrol**	**EL38: LL-UVB**	**Fold change (log2)**	**p-value**
Sugars and organic acids (2011/2012)								

Citric acid	0.24±0.42	0.22±0.33	0.13	0.00	0.21±0.19	0.18±0.13	0.22	0.00
Tartaric acid	8.55±0.08	8.56±0.08	0	0.00	7.39±0.17	6.84±0.05	0.11	0.20
Malic acid	1.67±0.33	1.37±0.36	0.29	0.00	2.61±0.2	2.07±0.37	0.33	0.70
Succinic acid	1.24±0.44	1.05±0.18	0.24	0.00	1.75±0.21	1.52±0.4	0.2	0.21
Glucose	113.84±0.08	112.89±0.05	0.01	0.00	106.32±0.07	102.44±0.06	0.05	0.25
Fructose	113.7±0.08	111.97±0.05	0.02	0.00	104.5±0.08	100.78±0.06	0.05	0.64
Photosynthetic pigments (2011/2012)								
Neoxanthin	53.65±0.89	238.71±0.37	-2.15	0.00	337.46±0.32	319.78±0.3	0.08	0.00
Violaxanthin		238.74±0.22		0.00	360.35±0.13	265.79±0.19	0.44	0.00
Lutein epoxide		195.56±0.22		0.00	399.37±0.05	183.12±0.1	1.12	0.00
Antheraxanthin	163.07±0.21	190.39±0.16	-0.22	0.01	182.52±0.21	159.08±0.13	0.2	0.15
Lutein	1180.17±0.2	1597±0.11	-0.44	0.00	1521.83±0.09	1355.68±0.16	0.17	0.00
Zeaxanthin	1979.12±0.25	1454.8±0.41	0.44	0.00	926.15±0.16	683.71±0.15	0.44	0.02
Chlorophyll b	2240.37±0.34	4428.22±0.33	-0.98	0.00	5129.41±0.1	4686.96±0.2	0.13	0.00
Chlorophyll a	6715.7±0.35	12037.08±0.26	-0.84	0.00	13936.62±0.09	12339.21±0.17	0.18	0.01
β-carotene	3235.4±0.18	6071.79±0.19	-0.91	0.00	6881.14±0.09	3725.71±0.2	0.89	0.00
Total chlorophylls	8956.07±0.35	16465.3±0.28	-0.88	0.00	19066.03±0.1	17026.17±0.18	0.16	0.01
Total carotenoids	6611.42±0.15	9987±0.13	-0.6	0.00	10608.82±0.08	6692.87±0.14	0.66	0.00
Total xanthophylls	3376.02±0.22	3915.2±0.15	-0.21	0.00	3727.68±0.11	2967.17±0.11	0.33	0.00
Chlorophyll a:Chlorophyll b (ratio)	3±0.07	2.79±0.09	0.1	0.00	2.72±0.02	2.64±0.04	0.04	0.23
Carotene:Chlorophyll (ratio)	0.4±0.36	0.38±0.18	0.07	0.50	0.36±0.05	0.22±0.18	0.71	0.00
β-Carotene+Lutein	4415.57±0.15	7668.79±0.17	-0.8	0.00	8402.97±0.08	5081.39±0.18	0.73	0.00
β-carotene+Lutein:Total Carotenoids (ratio)	0.67±0.07	0.77±0.07	-0.2	0.00	0.79±0.02	0.76±0.04	0.06	0.00
V+A+Z	2142.2±0.23	1883.93±0.31	0.19	0.00	1469.02±0.14	1108.58±0.1	0.41	0.00
DEPS (ratio)	1±0	0.86±0.08	0.22	0.00	0.75±0.05	0.76±0.06	-0.02	0.02
Lx:L (ratio)		0.12±0.25		0.00	0.26±0.1	0.14±0.16	0.89	0.00
Photosynthetic pigments (2014/2015)								
Neoxanthin	45.48±0.16	28.16±2.55	0.69	0.42	136.09±0.19	60.01±1.09	1.18	0.00
Violaxanthin	158.95±0.14	270.87±0.16	-0.77	0.00	216.99±0.15	288.86±0.12	-0.41	0.00
Lutein epoxide	101.8±0.08			0.00	77.62±0.13	9.65±3.46	3.01	0.00
Antheraxanthin	187.38±0.12	203.6±0.21	-0.12	0.26	179.45±0.16	184.8±0.14	-0.04	0.65
Lutein	1104.81±0.16	1076.7±0.12	0.04	0.66	1594.47±0.07	1337.53±0.1	0.25	0.00
Zeaxanthin	1822.17±0.13	1290.71±0.08	0.50	0.00	662.12±0.08	686.05±0.09	-0.05	0.32
Chlorophyll b	923.55±0.2	915.6±0.18	0.01	0.91	1999.19±0.08	1381.35±0.12	0.53	0.00
Chlorophyll a	2628.82±0.17	2343.31±0.16	0.17	0.10	4520.35±0.08	3351.72±0.12	0.43	0.00
β-carotene	468.57±0.14	368.53±0.19	0.35	0.00	721.39±0.08	550.44±0.12	0.39	0.00
Total chlorophylls	3552.37±0.18	3258.92±0.16	0.12	0.23	6519.54±0.08	4733.07±0.11	0.46	0.00
Total carotenoids	3889.16±0.1	3238.58±0.1	0.26	0.00	3588.14±0.04	3117.33±0.07	0.20	0.00
Total xanthophylls	3420.59±0.1	2870.05±0.09	0.25	0.00	2866.74±0.06	2566.9±0.08	0.16	0.00
Chlorophyll a:Chlorophyll b (ratio)	2.86±0.04	2.57±0.06	0.15	0.00	2.26±0.01	2.44±0.09	-0.11	0.01
Carotene:Chlorophyll (ratio)	0.13±0.12	0.11±0.11	0.20	0.00	0.11±0.05	0.12±0.1	-0.08	0.11
β-Carotene+Lutein	1573.38±0.14	1445.23±0.13	0.12	0.15	2315.86±0.05	1887.96±0.09	0.29	0.00
β-carotene+Lutein:Total Carotenoids (ratio)	0.4±0.09	0.45±0.07	-0.16	0.01	0.65±0.03	0.61±0.04	0.09	0.00
V+A+Z	2168.5±0.12	1765.19±0.08	0.30	0.00	1058.57±0.08	1159.71±0.07	-0.13	0.01
DEPS (ratio)	0.93±0.01	0.85±0.03	0.14	0.00	0.8±0.03	0.75±0.04	0.08	0.00
Lx:L (ratio)	0.09±0.15			0.00	0.05±0.17	0.01±3.46	2.69	0.00
Volatile compounds (2014/2015)								
β-ionone	10.93±0.34	6.43±0.23	0.77	0.00	11.48±0.16	12.94±0.32	0.00	0.29
β-Cyclocitral	2.43±0.24			0.00	2.44±0.1	1.9±0.63	0.00	0.14
β-Damascenone-69	3.81±0.19	3.87±0.11	-0.02	0.79	3.32±0.14	3.46±0.23	0.00	0.61
Geranylacetone	101.7±0.14	85.3±0.09	0.25	0.00	69.87±0.23	17.8±1.31	0.00	0.00
MHO	19.72±0.19	18.03±0.25	0.13	0.34	14.26±0.32	2.29±0.28	2.65	0.00
Limonene	37.8±0.11	18.42±0.22	1.04	0.00				0.00
α-Terpinene	17.4±0.25			0.00	1.15±3.46	1.25±3.46		0.96
Cineol								0.00
Gamα-Terpinene	48.67±0.23	17.86±0.64	1.45	0.00	3.6±2.35	1.27±3.46		0.41

α-Terpinolene	38.79±0.2	22.88±0.26	0.76	0.00				0.00
Linalool	80.71±0.18	36.85±0.28	1.13	0.00	8.56±0.1	6.87±0.27	0.00	0.01
α-Terpineol	6.63±0.31	3.99±0.22	0.73	0.00	1.18±0.26	0.8±0.43	0.00	0.01
Geraniol	4.8±0.29	1.78±0.17	1.43	0.00	1.44±0.2	2.46±1.09	0.00	0.20
4-Terpineol	0.47±0.77			0.00	0.05±3.46			0.33
Sabinene	6.49±1.26			0.01				0.00
Trans-2-Hexanal	7955.6±0.23	5253.91±0.36	0.60	0.00	7339.89±0.11	8503.3±0.18	-0.23	0.03
2-Hexanal	171.59±0.25	104.54±0.39	0.71	0.00	163.66±0.13	182.05±0.24	-0.17	0.21
N-Hexanal	894.66±0.48	348.63±0.41	1.36	0.00	621.14±0.17	1156.33±0.3	-0.90	0.00
1-Octen-3-one	44.57±0.21	58.42±0.13	-0.39	0.00	107.31±0.28	19.07±0.18	2.51	0.00
2-Heptanal	42.58±0.14	57.1±0.12	-0.42	0.00	86.8±0.28	19.5±0.18	2.18	0.00
Trans-2,4-Heptadienal	22.74±0.15	20.27±0.09	0.17	0.04	26.53±0.22	15.26±0.23	0.82	0.00
2-Octenal	33.27±0.11	39.82±0.12	-0.26	0.00	62.02±0.24	21.27±0.19	1.56	0.00
Octanal	3.34±0.19	3.46±0.12	-0.05	0.59	4.87±0.21	3.68±0.25	0.39	0.01
Nonenal	8.75±0.19	8.19±0.17	0.10	0.37	11.04±0.29	12.77±0.34	-0.27	0.27
3-Hexanol	1.04±0.79	2.25±0.12	-1.11	0.00	3.33±0.23	0.4±1.48	4.99	0.00
Hotrienol	171.44±0.19	127.18±0.19	0.43	0.00	45.96±0.21	8.21±3.04	0.40	0.00
Norisoprenoids	138.59±0.11	113.64±0.07	0.29	0.00	101.39±0.2	89.29±0.2	0.17	0.00
Mono-terpenes	235.27±0.13	101.77±0.3	1.21	0.00	15.99±0.56	11.06±0.1	0.41	0.35
Carbonyl compounds	9183.61±0.25	5894.34±0.34	0.64	0.00	8423.25±0.11	9933.23±0.18	-0.38	0.02
Alcohols	172.48±0.19	129.43±0.18	0.41	0.00	49.29±0.2	33.89±0.37	0.37	0.00
C6 compounds	9022.89±0.25	5709.32±0.35	0.66	0.00	8128.0±0.11	3844.81±0.2	1.08	0.01

Chapter 4

UVB attenuation impacts on berry amino acids and cell wall composition

4.1 Introduction

Light as a modulator of plant metabolism is well established, but detailed understanding of the impacts of light quality and quantity on primary and secondary metabolism, stress reactions as well as plant structure still requires attention. Here, an experimental design was implemented in a Sauvignon Blanc vineyard where UVB attenuation was achieved in both a high light (HL) and low light (LL) environment, leading to four distinct confirmed microclimates (refer to Chapter 3). In addition to the metabolite profiling that was already described in Chapter 3, the grape berry samples from the four unique microclimates were also subjected to amino acid analysis as well as cell wall compositional profiling. The rationale for these analyses stem from research that showed impacts of variable light exposure and/or UV effects on plant metabolism. The primary objective of this part of the study was to investigate the UVB specific responses of both a High Light (HL) and Low Light (LL) microclimate on amino acid profiles during the developmental stages of the berries, as well as the berry cell wall composition of ripe berries. Justification for these analyses are provided below.

Regarding nitrogen and consequently amino acid metabolism, both light exposure and UV radiation have been shown to elicit various responses, with most studies being conducted on vegetative tissues. In *Arabidopsis thaliana*, certain amino acids were shown to accumulate in leaf tissues under light conditions, while other amino acids accumulated in the dark (Coruzzi et al., 2015). Similarly, dark conditions induced an accumulation of asparagine in several plant tissues including wheat (Peeters and Laere, 1992) and pea (Joy et al., 1983). Noctor et al. (1997) furthermore demonstrated a light-induced accumulation in glutamine, asparagine, serine and glycine pools in poplar tree leaves. In grapevine specifically, a recent study conducted on cluster shading in two grape cultivars (Gamay Noir and Gamay Fréaux) showed that an attenuation in light exposure reduced the general amino acid content of ripe berries, with a greater response occurring in the skin tissues compared to the berry pulp. Furthermore, the response differed between the two cultivars, with Gamay Noir exhibiting a significantly stronger reduction in amino acids with light attenuation. This study therefore showed that the amino acid content of ripe berries is modulated by light quantity and that the reaction is specific to tissue type and grape cultivar (Guan et al., 2017). Similarly, another trial demonstrated that an increase in the light exposure of grape berries resulted in a higher accumulation of certain amino acids, including valine, leucine, and serine, as well as the stress-related proline and GABA (γ-aminobutyric acid), measured at the ripe stage of development in Cabernet Sauvignon (Reshef et al., 2017). Furthermore, a recently published paper from our own research group highlighted the importance of amino acids as potential sources of energy

for stress tolerance mechanisms under increasing light exposure. Furthermore, it was clear that the acclimation of the berries to different light exposures activated the accumulation/depletion of different metabolites in the early and late developmental stages (du Plessis et al., 2017).

The particular influences of light quality on amino acid metabolism in grape berries have received little attention in the past and the few results reported are not always in support of each other. Schultz et al. (1998) demonstrated that reduced UVB radiation increased the content of arginine and glutamine in Riesling grape berries at the harvest stage, while Gregan et al. (2012) stated that UVB attenuation did not significantly affect the majority of amino acids in Sauvignon Blanc. Keller and Torres-Martinez (2004) showed that the influence of UV varied amongst the individual amino acids with proline and arginine remaining unaffected in both Chardonnay and Cabernet Sauvignon mature berries. Martínez-Lüscher et al. (2014) also showed that the total free amino acid concentration was unaffected by increased UVB in Tempranillo berries, but certain amino acids responded to the higher radiation, particularly GABA which was shown to increase. Despite the limited research and discrepancies in results, these studies reiterate the premise that the metabolic profile of grape berries is modulated by variations in light quality, supporting the need for further study on UVB radiation impacts.

Furthermore, a number of studies have highlighted the significance of light signals in plant cell wall physiology. Light is involved in the optimisation of leaf light interception by facilitating directional cell expansion in shade conditions (Gall et al., 2015; Sasidharan et al., 2008, 2010, 2014) and inhibiting shoot elongation with increased light exposure (Kigel and Cosgrove, 1991; Masuda et al., 1981). It has furthermore been demonstrated that lignin biosynthesis may be influenced by light quantity and quality as their production occurs via the phenylpropanoid and monolignol pathways, both of which are influenced by variations in light exposure or UV radiation (Cabane et al., 2012; Schopfer et al., 2001; Kimura et al., 2003; Hilal et al., 2007). In pear fruit, it was demonstrated that high light exposure during ripening led to increased firmness at harvest as they displayed a larger average molecular size of tightly bound glycans and a delayed pectin solubilisation related to the reduced removal of RG I-arabinan side chains (Raffo et al., 2011). Post-harvest irradiation with UVC has also been shown to prolong fruit firmness by modifying enzyme activity (Pombo et al., 2009).

In addition to the possible impacts of UV and/or increased light exposure on the berry cell wall structures, the cell wall physiology is directly related to the extractability of certain organoleptically beneficial compounds. The focus on cell wall composition of the ripe grapes in this study stems from the perspective that possible changes to the cell walls of the berry tissues could ultimately influence the release of compounds during winemaking. Since many of the compounds previously found to respond to the light exposure and/or UV attenuation are (wine) quality-impact factors that are known to be distributed between different tissue types in the berries, the cell walls of the ripe berry skins and pulp

cells were investigated. Very little information on grape berry cell walls exists, specifically in context of stress impacts, however some trials have alluded to potential UV impacts. For example, a study conducted on grapevine cells cultured *in vitro* showed that UVC light reduced cell wall elasticity due to physical modifications to the cell wall structure. A similar result was seen in a comparative study where the cells were infected with *Botrytis cinerea*, suggesting that the diminished cell wall elasticity is a general stress response and aids in plant defence (Lesniewska et al., 2004). Cell wall profiling methods, such as Comprehensive Microarray Polymer Profiling (CoMPP) and monosaccharide analysis have proven useful in such studies in profiling cell wall composition and architecture, supporting plant cell wall research and the investigation of potential impacts of abiotic stress (Gao et al., 2015, 2016a; Moore et al., 2014a; Zietsman et al., 2015).

4.2 Materials and Methods

4.2.1 Vineyard treatments

The same vineyard and experimental layout was used as is described in Chapter 3. Leaf removal in the bunch zone (at EL 29) and the installation of UVB attenuating acrylic sheets (Perspex® South Africa) created four different microclimates from which berries were sampled. These included a HLcontrol and HL-UV and well as a LLcontrol and LL-UVB environment. The experiment was conducted over three seasons, but the berry amino acid data and berry cell wall analysis presented was prepared from samples from the third (2014/2015) season.

4.2.2 Amino acid analysis

Sampling of the berries occurred at three important phenological stages, namely, berry pea-size (EL31), véraison (EL34), and the ripe stage (EL38). Sampling was conducted as described in Chapter 3 and frozen immediately in the field after being picked using liquid nitrogen. The berry tissue was milled after the seeds were removed and the samples were then stored at -80°C until analysis. The whole berry amino acids (skin and pulp combined) were analysed with HPLC according to the method described in du Plessis et al. (2017). This was done for each of the three developmental stages with four biological repeats per treatment and three technical repeats per sample.

4.2.3 Berry cell wall analysis

4.2.3.1 Berry sampling and preparation

Sampling was carried out at the ripe stage of berry development (EL-38) and consisted of two biological repeats. For each repeat, a pooled representative sample of three bunches was taken randomly from the vineyard over all experimental panels form each of the four microclimates (refer to Figure 4.1). This means that a total of six bunches were sampled for each of the four microclimates. The bunches were kept cold and transported back to the laboratory for processing. Berries were removed from the rachis and only berries from the exposed side of the selected bunches were selected. These berries were then peeled, the seeds removed and the skin and pulp tissue immediately frozen separately in liquid nitrogen. This was done in triplicate with each repeat consisting of a minimum of 30 berries (Figure 4.1). The tissue was then milled in liquid nitrogen and stored at -80°C until analysis.

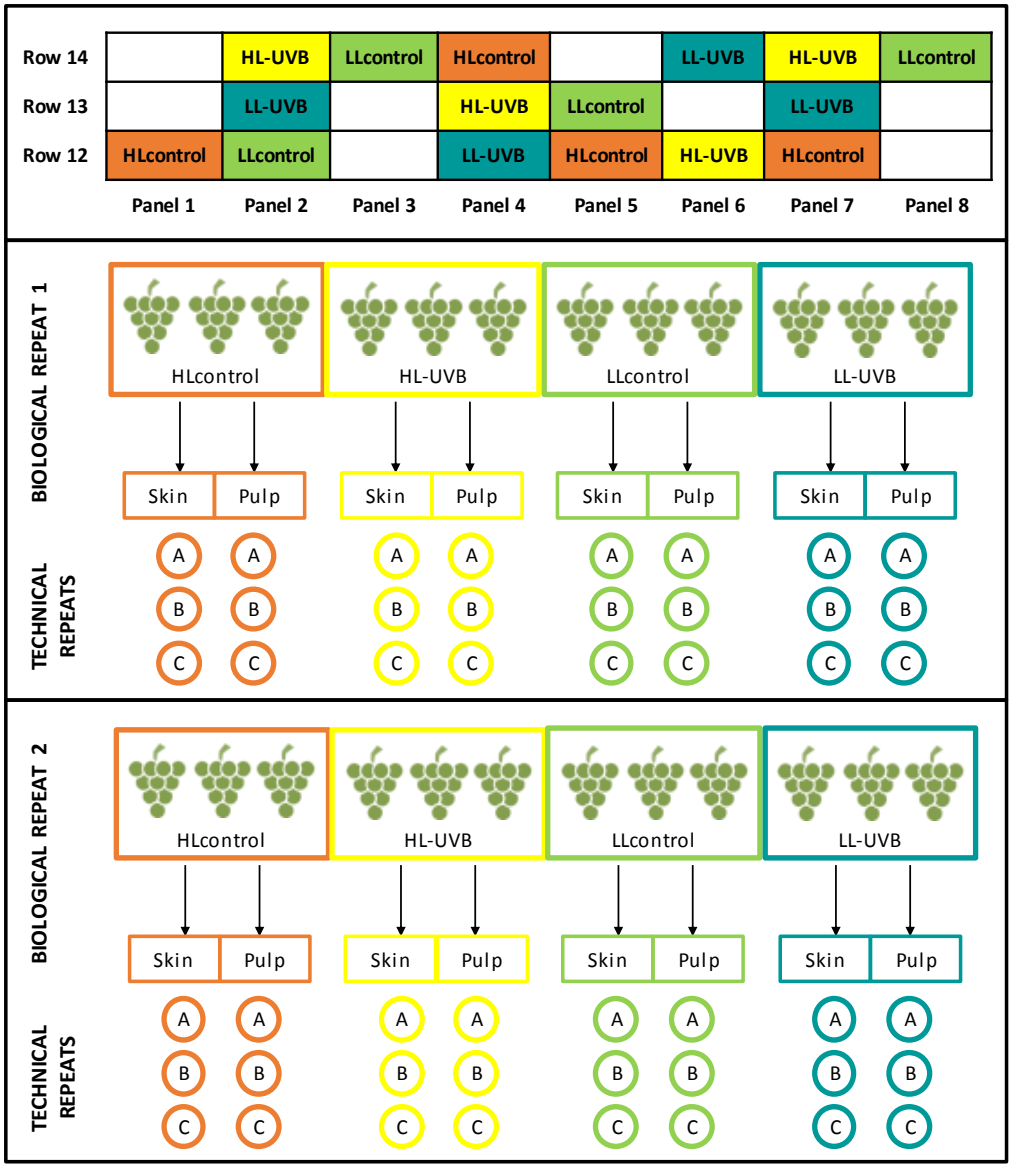


Figure 4.1. Ripe berry sampling for cell wall analysis of skin and pulp tissues. Grapes were pooled for each microclimate from which two biological repeats were randomly selected. Samples were split into skin and pulp fractions. Sufficient tissue was collected from each biological repeat for three technical repeats.

4.2.3.2 Extraction of alcohol insoluble fraction (AIR) from berry tissues

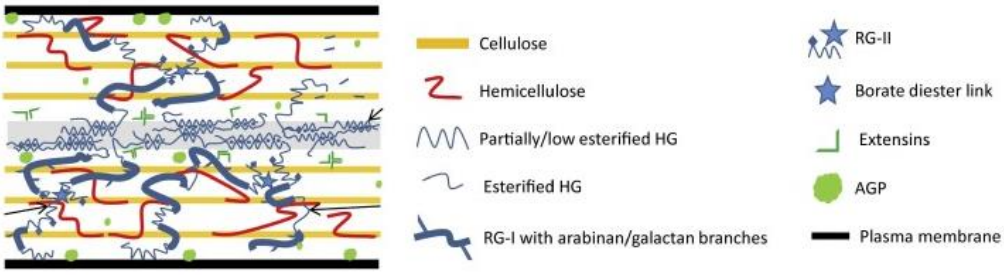
The alcohol insoluble fraction (AIR) used for cell wall compositional analysis was isolated from homogenised tissue berry skin and pulp tissue using the method described in (Ortega-Regules et al. (2008) using the modifications outlined in Zietsman et al. (2017).

4.2.3.3 CoMPP and monosaccharide composition analysis of berry cell wall material

Comprehensive Microarray Polymer Profiling (CoMPP) analysis of the AIR fraction was conducted in Denmark (Institute of Molecular Biology, University of Copenhagen, Denmark). The tissue was sequentially extracted to obtain the pectin and hemicellulose rich fractions using CDTA (diamino-

cyclo-hexane-tetra-acetic acid) and then NaOH respectively. The arrays printed with these fractions were then probed with a panel of monoclonal antibodies (mAbs) and carbohydrate binding modules (CBMs) (Moller et al., 2007; Moore et al., 2014a). The target specific epitopes related to cell wall polymers. This method was described in Moller et al. (2007) and has been validated for grapevine leaf (Moore et al., 2014b) and berry tissue (Moore et al., 2014a). A list of the probes used in the analysis is provided in Table 4.1, with an indication of how these probes correspond to the different cell wall polymers. A mean spot signal was calculated and each of the arrays was normalised to the highest signal in the dataset. A cut-off value of 5 was instated whereby any values calculated to be lower than 5 were said to be zero. The monosaccharide composition of the AIR fractions was also determined using GC-MS as described in Zietsman et al. (2015) and expressed as pM/mg AIR. Two representative samples per tissue type per treatment were selected for this analysis.

Table 4.1. A list of the probes used in the CoMPP analysis. A representation of the cell wall is presented and the probes and their targets are shown in relation to the cell wall structural model. Different colour components correlate with the coloured text in the table to give an indication of the cell wall components targeted by the different antibodies (Adapted from Zietsman et al., 2017).



Monoclonal antibody	Reference
HG (homogalacturonan) partially/de-esterified (mAb JIM5)	(Verhertbruggen et al., 2009)
HG partially esterified (mAb JIM7)	(Verhertbruggen et al., 2009)
HG partially/de-esterified (mAb LM18)	(Verhertbruggen et al., 2009)
HG partially/de-esterified (mAb LM19)	(Verhertbruggen et al., 2009)
HG partially esterified (mAb LM20)	(Verhertbruggen et al., 2009)
HG Ca^{2+} dimers (mAb 2F4)	(Liners et al., 1989)
RG-I (rhamnogalacturonan-I), 6 unbranched disaccharide (mAb INRA-RU1)	(Ralet et al., 2010)
RG-I, 2 unbranched disaccharide (mAb INRA-RU2)	(Ralet et al., 2010)
α-1,4-D-galactan (mAb LM5)	(Jones et al., 1997)
α-1,5-L-arabinan (mAb LM6)	(Willats et al., 1998)
Linearized α-1,5-L-arabinan (mAb LM13)	(Moller et al., 2008)
β-1,4-D-(galacto)(gluco)mannan (mAb LM21)	(Marcus et al., 2010)
β-1,4-D-(gluco)mannan (mAb LM22)	(Marcus et al., 2010)
β-1,3-D-glucan (mAb BS-400-2)	(Moller et al., 2008)
Xyloglucan (XXXG motif) (mAb LM15)	(Marcus et al., 2010)
Xyloglucan (mAb LM25)	(Pedersen et al., 2012)

β-1,4-D-Xylan (mAb LM10)	(McCartney et al., 2005)
β-1,4-D-Xylan d/arabinoxylan (mAb LM11)	(McCartney et al., 2005)
Cellulose (crystalline) (mAb CBM3a)	(Blake et al., 2006)
Extensin (mAb LM1)	(Neumetzler et al., 2012)
Extensin (mAb JIM11)	(Smallwood et al., 1994)
Extensin (mAb JIM20)	(Smallwood et al., 1994)
AGP (arabinogalactan proteins) (mAb JIM8)	(Pennell et al., 1991)
AGP (mAb JIM13)	(Yates et al., 1996)
AGP (mAb LM14)	(Moller et al., 2008)
AGP,b-linked GlcA (mAb LM2)	(Yates et al., 1996)

4.2.4 Data analysis

Microsoft Excel and Statistica (version 12) were utilised for standard statistical analysis and the multivariate analysis was conducted using SIMCA (version 14 from Sartorius Stedim Data Analytics AB).

4.2.4.1 Amino acid data analysis

The amino acid data sets were statistically evaluated and subjected to multivariate data analysis. The data from all the microclimates was analysed using OPLS-DA models using the three main factors (i.e. development, light exposure or UVB attenuation) as Y-variables. The use of supervised OPLS-DA models assisted in the visualisation of the complex datasets, which consisted of multiple variables and helped to identify putative correlations within the dataset. The practicalities of these models are described in Chapter 3. This data was further validated using a repeated measures ANOVA. This was done in order to rank the significance of each compound in response to the three main experimental factors (i.e. development, light exposure and UVB attenuation) individually and in combination. This analysis allowed for the testing of potential cause-effect relationships between the measured compounds and the main experimental factors.

Further multivariate analysis was conducted to test the responses of the amino acids to the individual treatments on a "per developmental stage" basis. Initially, the influence of light exposure was examined per stage by comparing the HLcontrol and LLcontrol samples. Thereafter, UVB responses were investigated per stage for both light environments by comparing the HLcontrol/HL-UVB and LLcontrol/LL-UVB contrasts. This was done initially using OPLS-DA models. Further statistical evaluation was conducted using factorial ANOVAs. Significance was tested using Fisher LSD Post Hoc tests to confirm which compounds reacted statistically significantly to the specified factors (adjusted p-value, q-value). Linear models were also fitted to the contrasts showing significant variation in order to visualise the actual contents of the relevant compounds during berry development.

4.2.4.2 Cell wall data analysis

The normalised data from the CoMPP analysis was used to construct the different heatmaps. The raw data was also tested statistically for significance using factorial ANOVA plots and Fisher LSD post hoc tests (adjusted p-value, q-value). Furthermore, the differences between the HLcontrol and HL-UVB as well LLcontrol and LL-UVB data points were calculated and a value above 10 was set to indicate biological significance between the controls and UVB attenuation treatments under the HL and LL microclimates. Monosaccharide data was initially tested using basic t-tests, but was also subjected to a factorial ANOVA and then further tested using Fisher LSD Post Hoc analysis to confirm which signals were statistically significantly different in each contrast (adjusted p-value, q-value).

4.3 Results

4.3.1 Amino acid levels in the berry samples from the four microclimates

Twenty two amino acids were detected in all berry stages, with clear differences in amino acid levels amongst the three developmental stages tested, but also with regards to the four microclimates (See Supplementary Table 3.4 for the complete amino acid dataset).

All the amino acid data was firstly analysed with OPLS-DA plots using developmental stage (Figure 4.2A), light exposure (Figure 4.2B) or UVB attenuation (Figure 4.2C) as Y- variables. Interestingly in both the light exposure and UVB attenuation models, the developmental stages were still clearly separated, highlighting development as a driving variable in berry amino acid metabolism. Most notably, the total amino acid pool was strongly associated with the early developmental stage. Separation was also seen between the samples with a variation in light exposure, confirming its influence on the amino acids. The amino acids which contributed most significantly to the model are presented in the corresponding coefficient plot in Figure 4.2B. Regarding UVB attenuation, the separation in the model between samples was less clear, however the corresponding coefficient plot (Figure 4.2C) highlighted certain amino acids including glutamine, arginine, asparagine, leucine and isoleucine as major contributors to the separation.

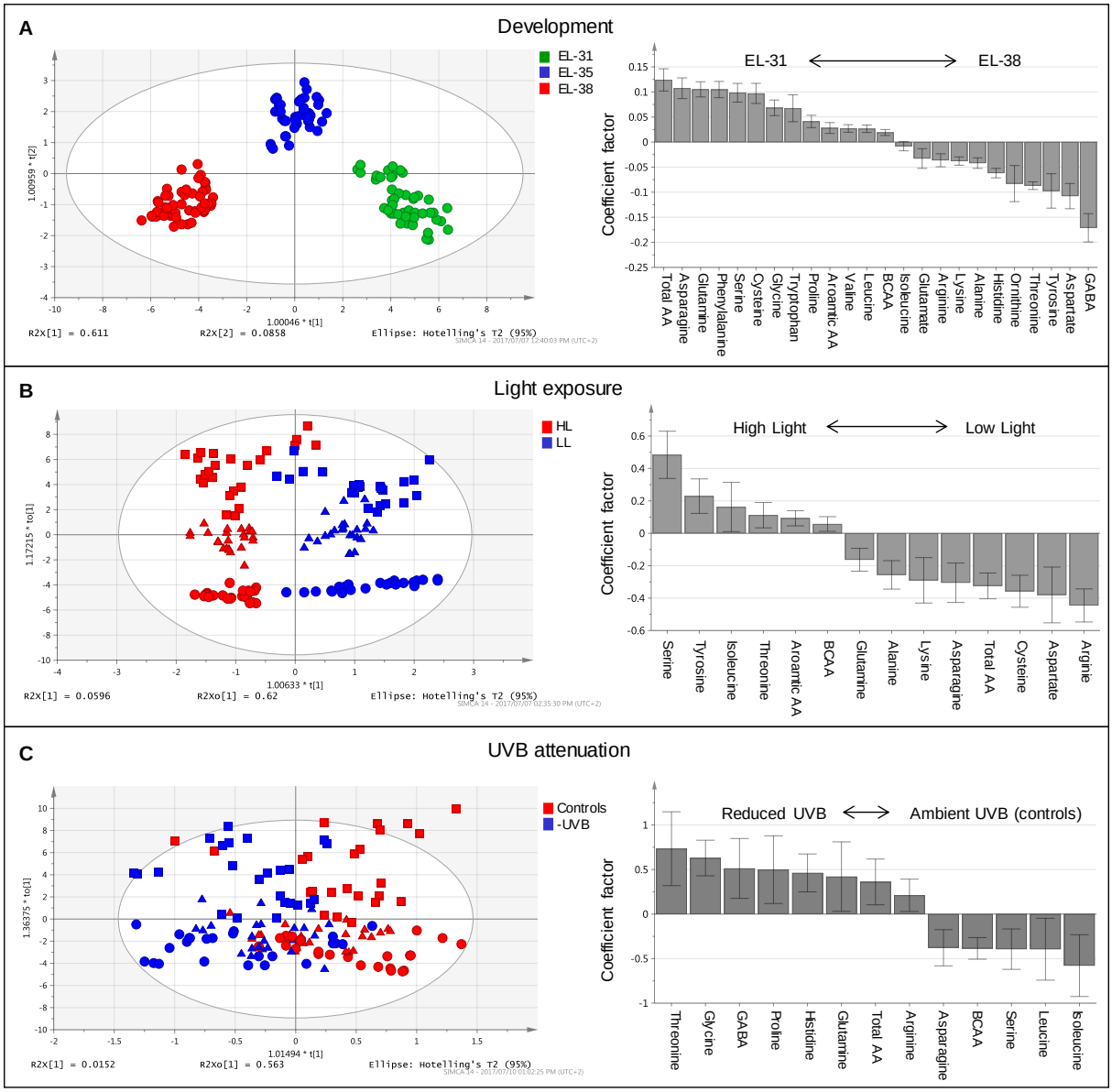


Figure 4.2. OPLS-DA models generated for all metabolic data over both experimental seasons for developmental stage (A) light exposure (B) and UVB attenuation (C). Each OPLS-DA is accompanied by a co-efficient plot of compounds which contributed most to the respective models. These were chosen according to the individual variable importance plots (VIP's) and included the top compounds with a value above 0.9. Shapes of the sample icons in B and C denote the respective developmental stages: EL-31 (●), EL-35 (▲) and EL-38 (■).

The results of the OPLS-DA plots were further statistically investigated and validated using a repeated measures ANOVA in order to rank the significance of each compound in response to the three main experimental factors (i.e. development, light exposure and UVB radiation) individually, and in combination (Table 4.2). The results confirmed that developmental stage was the most significant driver, followed by the variation in light exposure. Interestingly UVB attenuation only elicited a significant effect in combination with developmental stage and light exposure.

Table 4.2. An analysis of the amino acids (2014/2015 season). The repeated measures ANOVA results for the listed parameters and individual compounds are reported as F-values. Values are scaled from highest (i.e. most significant) to lowest by colour. Green indicates low F-values, while red indicates high F-values values. All insignificant values (F≤3) are coloured in grey. Different superscripted letters indicate significant differences between variables: p-value < 0.001 [a]; 0.001< p-value < 0.01[b]; 0.01 < p-value < 0.05[c] and insignificant [d] Maximum ■ ; 50% ■ ; minimum ■ ; insignificant ■ .

	Development	Exposure	UVB-attenuation	Exposure × Development	UVB-attenuation × Development	UVB-attenuation × Exposure	UVB-attenuation × Exposure × Development
Leucine (Leu)	433.15[a]	11.39[b]	0.14[d]	10.27[a]	0.42[d]	2.06[d]	3.46[c]
Isoleucine (Ile)	403.45[a]	31.34[a]	0.25[d]	13.47[a]	3.43[c]	1.01[d]	3.78[c]
Proline (Pro)	352.83[a]	27.79[a]	1.07[d]	14.47[a]	0.34[d]	1.58[d]	0.57[d]
Glutamate (Glu)	312.62[a]	3.22[d]	0.67[d]	30.03[a]	0.34[d]	0.08[d]	6.69[b]
Glutamine (Gln)	288.31[a]	47.24[a]	0.01[d]	33.10[a]	0.22[d]	0.07[d]	0.41[d]
Alanine (Ala)	273.39[a]	0.01[d]	0.01[d]	6.43[b]	1.42[d]	1.16[d]	2.52[d]
Threonine (Thr)	271.49[a]	4.45[d]	0.85[d]	2.47[d]	0.28[d]	0.87[d]	2.38[d]
Phenylalanine (Phe)	259.00[a]	1.25[d]	0.38[d]	6.67[b]	0.84[d]	0.00[d]	3.76[c]
Tryptophan (Trp)	254.03[a]	5.26[c]	0.08[d]	14.07[a]	0.31[d]	0.74[d]	3.02[d]
Arginine (Arg)	210.35[a]	9.61[b]	0.08[d]	4.14[c]	0.68[d]	0.98[d]	0.24[d]
Asparagine (Asn)	176.77[a]	49.72[a]	0.70[d]	12.51[a]	0.18[d]	1.64[d]	0.61[d]
Lysine (Lys)	116.76[a]	0.00[d]	0.00[d]	8.54[b]	8.36[b]	0.73[d]	4.30[c]
Tyrosine (Tyr)	90.76[a]	6.13[c]	0.00[d]	36.72[a]	0.70[d]	0.51[d]	2.28[d]
Valine (Val)	90.14[a]	4.38[d]	0.01[d]	22.02[a]	2.20[d]	1.68[d]	8.14[b]
Cysteine (Cys)	89.04[a]	11.14[b]	0.09[d]	6.29[b]	1.34[d]	2.11[d]	1.05[d]
Histidine (His)	86.93[a]	0.23[d]	0.02[d]	2.79[d]	0.26[d]	1.57[d]	0.63[d]
GABA	71.69[a]	3.08[d]	2.34[d]	3.03[d]	0.47[d]	5.29[c]	5.67[b]
Serine (Ser)	41.22[a]	0.60[d]	0.56[d]	0.48[d]	0.75[d]	0.68[d]	9.43[a]
Aspartate (Asp)	26.92[a]	14.97[b]	0.36[d]	0.75[d]	1.86[d]	0.13[d]	0.85[d]
	Development	Exposure	UVB-attenuation	Exposure × Development	UVB-attenuation × Development	UVB-attenuation × Exposure	UVB-attenuation × Exposure × Development
Branched chain amino acids (BCAA)	300.60[a]	10.86[b]	0.01[d]	24.79[a]	2.81[d]	2.07[d]	9.07[b]
Aromatic amino acids (AA)	123.50[a]	2.51[d]	0.04[d]	38.19[a]	1.69[d]	1.01[d]	9.63[a]
Total amino acids (AA)	121.18[a]	27.53[a]	0.04[d]	35.54[a]	0.21[d]	0.01[d]	1.58[d]

4.3.3 The responses of amino acids to light and UVB attenuation

Initially, to further investigate the effects of light incidence on amino acid metabolism, OPLS-DA plots were created for the early and late developmental stages separately using light exposure as the Y-variable. These results were statistically validated using ANOVA and Fisher LSD post hoc tests and it was seen that different amino acids were responsive to light exposure in the two developmental stages. These results are presented in Supplementary Figure 4.1.

To better elucidate the subtle effects of UVB attenuation, OPLS-DA plots were created for the HL and LL microclimates separately in the early and late stages of development. Those amino acids which had a value >0.9 on the corresponding VIP plots were listed. These results were statistically validated and post hoc analysis highlighted the amino acids which responded significantly to UVB attenuation in each stage. In the green stage (EL-31), a number of amino acids were listed on the corresponding VIP plots as driving factors of the separation in the models, however none were statistically significant according to the post hoc tests (Supplementary Figure 4.2A and B). At harvest (EL-38), different amino acids drove the separation in the HL and LL OPLS-DA models. In the HL microclimate, several amino acids responded statistically significantly to the attenuation of UVB with all of them being associated with the control (Supplementary Figure 4.3A). In the LL microclimate, only GABA showed a statistically significant difference and was seen to be associated with UVB attenuation (Supplementary Figure 4.3B). It is clear form these models and the repeated measures ANOVA (Table 4.2) that the effects of UVB attenuation on the amino acids are dependent on both the developmental stage and the light environment.

4.3.3.1 In the ripe berry stages specific amino acids responded to UVB attenuation

The most noteworthy amino acid responses were seen in the late developmental stage (EL-38) in both the HL and LL environments. These results were further statistically evaluated for significance, highlighting particularly important amino acids (Figure 4.3). In the HL microclimate, UVB attenuation strongly reduced the levels of the branched chain amino acids, namely valine, leucine and isoleucine as well as alanine and GABA. Furthermore, in addition to the general lower levels of amino acids observed in the LL microclimates, UVB attenuation led to an increase in GABA in the LL-UVB samples, reaching a similar level seen in the HL-UVB samples. No further UVB influence was noted in the LL microclimate.

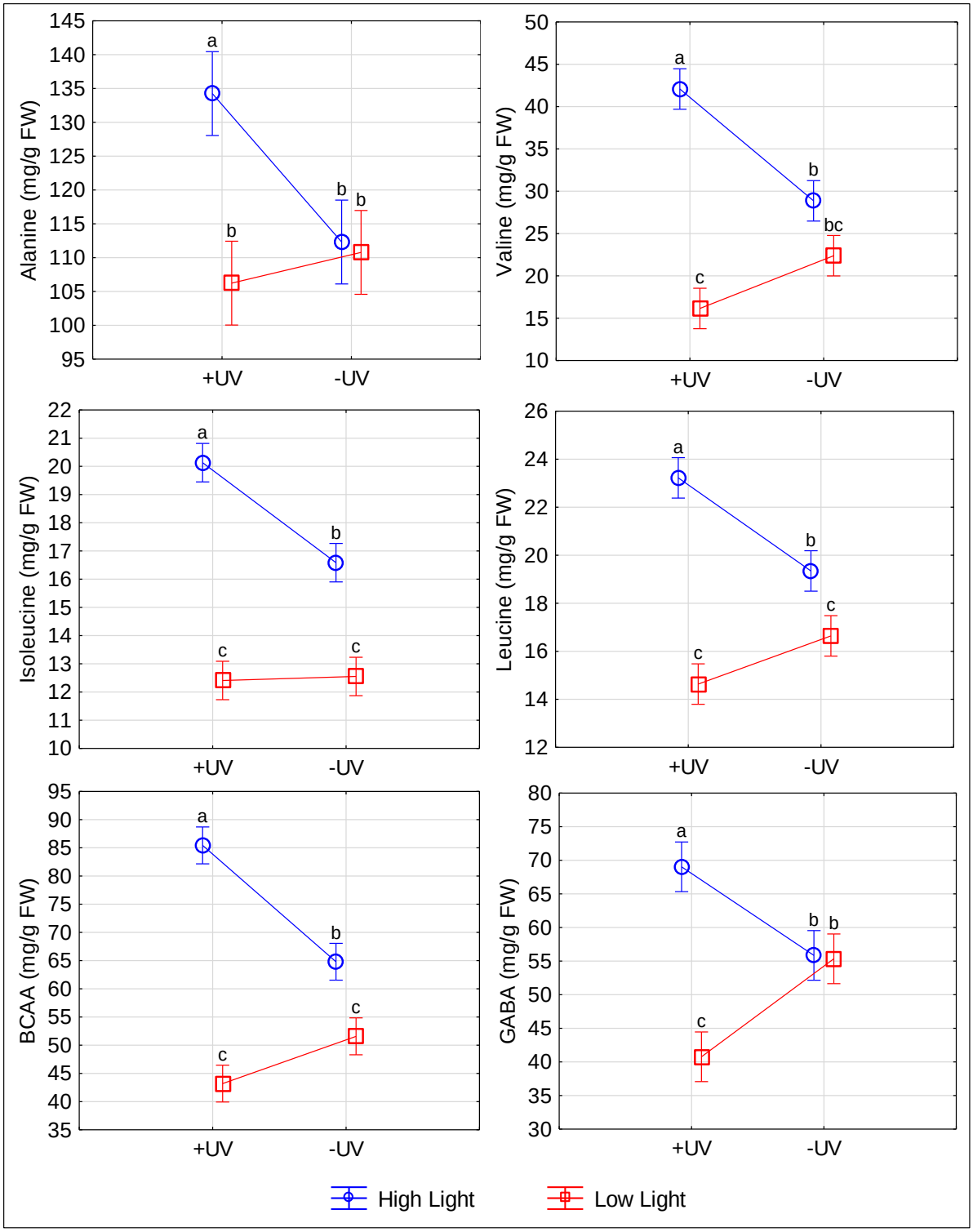


Figure 4.3. The ANOVA plots for selected amino acids and amino acid pools measured in the ripe berries (EL38). The results for both high light and low light environments are represented. Different letters indicate significant difference according to Fischer LSD post hoc tests (q-value, adjusted p-value).

4.3.4 Cell wall analysis of ripe berry skin and pulp tissues

The CoMPP analysis included the sequential extraction of the pectin and hemicellulose rich fraction using CDTA and NaOH respectively. The epitope binding profiles are similar to what is known for grape berries (Gao et al., 2015; Moore et al., 2014a; Zietsman et al., 2015). Profiling data of the skin and pulp cell wall samples (Table 4.3) showed high levels of homogalacturonans (HG) and rhamnogalacturonan with its associated side chains extracted from the CDTA fraction with some extensins and AGPs. These levels were generally higher in the pulp tissue as opposed to the skin tissue, with a few exceptions seen in specific epitopes. Additionally, strong signals for the epitopes linked to glucans, xyloglucans, cellulose, extensins and AGPS were seen in the hemicellulose rich fraction (NaOH) as well as epitopes linked to rhamnogalacturonan with its associated side chains, particularly in the pulp tissue. No significant differences were however observed between the grapes from the four microclimates. To support the CoMPP profiling analysis, the AIR from the samples was also subjected to monosaccharides analysis (Table 4.4) which confirmed that the samples did not differ significantly between the controls and UVB attenuation samples in either the HL or LL microclimate.

Table 4.3. A heatmap showing relative CoMPP data from the HLcontrol, HL-UVB, LLcontrol and LL-UVB samples for the EL38 berry skin and pulp tissue for both extraction fractions.

Extraction	Tissue	Treatment	HG						RG1 and side chains					Mannans		Glucans/ Xyloglucans			Xylans		Cellulose	Extensins			AGP's			
			HG partially/de-esterified (mAb JIM5)	HG partially esterified (mAb JIM7)	HG partially/de-esterified (mAb LM18)	HG partially/de-esterified (mAb LM19)	HG partially esterified (mAb LM20)	HG Ca2+ dimers (mAb 2F4)	RG-I, 6 unbranched disaccharide (mAb INRA-RU1)	RG-I, 2 unbranched disaccharide (mAb INRA-RU2)	b-1,4-D-galactan (mAb LM5)	a-1,5-L-arabinan (mAb LM6)	Linearised a-1,5-L-arabinan (mAb LM13)	b-1,4-D-(galacto)(gluco)mannan (mAb LM21)	b-1,4-D-(gluco)mannan (mAb LM22)	b-1,3-D-glucan (mAb BS-400-2)	Xyloglucan (XXXG motif) (mAb LM15)	Xyloglucan (mAb LM25)	b-1,4-D-Xylan (mAb LM10)	b-1,4-D-Xylan d/arabinoxylan (mAb LM11)	Celulose (crystalline) (mAb CBM3a)	Extensin (mAb LM1)	Extensin (mAb JIM11)	Extensin (mAb JIM20)	AGP (mAb JIM8)	AGP (mAb JIM13)	AGP (mAb LM14)	AGP,b-linked GlcAb (mAb LM2)
CDTA	Skin	HLcontrol	52	77	29	30	68	16	25	7	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	14	0	10
CDTA	Skin	HL-UVB	58	79	37	36	70	24	27	9	0	8	0	0	0	0	0	0	0	0	0	0	0	0	0	13	0	12
CDTA	Skin	LLcontrol	51	73	28	27	66	15	23	7	0	6	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	11
CDTA	Skin	LL-UVB	53	75	30	31	67	18	25	9	0	7	0	0	0	0	0	0	0	0	0	0	0	0	0	12	0	9
CDTA	Pulp	HLcontrol	33	81	24	21	75	6	54	45	10	35	14	0	0	0	0	0	0	0	0	22	16	24	0	15	0	0
CDTA	Pulp	HL-UVB	36	91	28	27	82	11	60	52	11	41	18	0	0	0	0	0	0	0	0	29	18	32	0	16	0	0
CDTA	Pulp	LLcontrol	33	83	28	24	77	11	57	50	13	39	15	0	0	0	0	0	0	0	0	31	20	35	0	18	0	0
CDTA	Pulp	LL-UVB	27	90	25	25	85	12	63	57	15	42	17	0	0	0	0	0	0	0	0	31	20	32	7	20	0	6
NaOH	Skin	HLcontrol	0	0	0	0	0	0	14	0	26	0	0	46	22	0	70	66	0	0	71	13	21	18	9	18	6	0
NaOH	Skin	HL-UVB	0	0	0	0	0	0	17	0	19	0	0	42	16	0	69	66	0	0	67	17	24	22	9	19	8	0
NaOH	Skin	LLcontrol	0	0	0	0	0	0	14	0	18	0	0	42	15	0	68	65	0	0	64	15	24	22	9	20	8	0
NaOH	Skin	LL-UVB	0	0	0	0	0	0	15	0	20	0	0	39	14	0	67	64	0	0	65	12	21	19	8	18	6	0
NaOH	Pulp	HLcontrol	0	0	0	0	0	0	50	41	20	41	27	35	0	13	49	43	0	0	55	34	25	26	19	29	13	7
NaOH	Pulp	HL-UVB	0	0	0	0	0	0	48	40	19	41	27	35	6	10	48	43	0	0	56	37	26	28	19	28	14	8
NaOH	Pulp	LLcontrol	0	0	0	0	0	0	51	43	22	43	28	33	0	15	48	43	0	0	50	33	29	29	22	32	15	10
NaOH	Pulp	LL-UVB	0	0	0	0	0	0	40	31	19	31	19	27	0	14	43	40	12	17	43	33	28	27	20	31	13	9

Table 4.4. Calculated means ± standard deviation of measured monosaccharides for the four microclimates expressed as micromolar/mg AIR. Pairwise t-tests was conducted to test for significance between the HLcontrol/ HL-UVB LLcontrol/LL-UVB treatments for skin and pulp tissue in EL38 berries, p- values < 0.05 are highlighted in red. The adjusted p-values (q-values) determined by factorial ANOVA and Fisher LSD post hoc tests however showed no significant differences. The superscripted "NS" denotes this.

	Skin					
	HLcontrol	**HL-UVB**	**p-value**	**LLcontrol**	**LL-UVB**	**p-value**
Arabinose	0.14±0.04	0.15±0.02	0.75^{NS}	0.18±0.03	0.14±0.02	0.29^{NS}
Rhamnose	0.04±0	0.04±0	0.54^{NS}	0.04±0	0.04±0	0.51^{NS}
Fucose	0.02±0	0.03±0	0.43^{NS}	0.03±0	0.03±0	0.39^{NS}
Xylose	0.07±0.01	0.07±0	0.67^{NS}	0.08±0.01	0.07±0.01	0.32^{NS}
Galacturonic acid	0.65±0.2	0.67±0.06	0.92^{NS}	0.86±0.16	0.48±0.18	0.16^{NS}
Mannose	0.02±0	0.02±0	0.79^{NS}	0.03±0.01	0.03±0.01	0.89^{NS}
Galactose	0.08±0.02	0.08±0.01	0.84^{NS}	0.09±0.02	0.07±0.02	0.54^{NS}
Glucose	0.08±0	0.1±0.01	0.07^{NS}	0.09±0.01	0.1±0.05	0.78^{NS}
Glucuronic acid	0.05±0.02	0.09±0.07	0.52^{NS}	0.08±0	0.05±0.01	0.04^{NS}
Total sugars	1.14±0.31	1.24±0.08	0.7^{NS}	1.48±0.24	1.01±0.14	0.14^{NS}
	Pulp					
	HLcontrol	**HL-UVB**	**p-value**	**LLcontrol**	**LL-UVB**	**p-value**
Arabinose	0.19±0.01	0.22±0	0.03^{NS}	0.21±0.02	0.19±0.02	0.39^{NS}
Rhamnose	0.05±0	0.05±0	0.73^{NS}	0.05±0	0.05±0	0.87^{NS}
Fucose	0.03±0	0.03±0	0.43^{NS}	0.03±0	0.03±0	0.78^{NS}
Xylose	0.08±0	0.07±0.03	0.57^{NS}	0.09±0.01	0.08±0.01	0.87^{NS}
Galacturonic acid	0.69±0.02	0.55±0.34	0.6^{NS}	0.68±0.06	0.8±0.07	0.2^{NS}
Mannose	0.02±0	0.02±0.01	0.89^{NS}	0.02±0	0.02±0	0.77^{NS}
Galactose	0.12±0.01	0.12±0.03	0.99^{NS}	0.13±0.01	0.13±0.01	0.82^{NS}
Glucose	0.05±0.01	0.05±0.03	0.75^{NS}	0.06±0	0.05±0	0.06^{NS}
Glucuronic acid	0.07±0	0.04±0.02	0.2^{NS}	0.07±0.01	0.06±0.01	0.39^{NS}
Total sugars	1.3±0.04	1.13±0.46	0.65^{NS}	1.34±0.11	1.41±0.04	0.48^{NS}

Taking into consideration the amino acid responses seen in this study as well as the cell wall analyses, the model of UVB responses on grape processes presented in chapter 3 was extended to include the most important UVB results generated here. Figure 4.4 therefore summarises the UVB responses and acclimation strategies employed in grape berries under HL and LL conditions in the green and ripe berry tissues presented in these two Chapters.

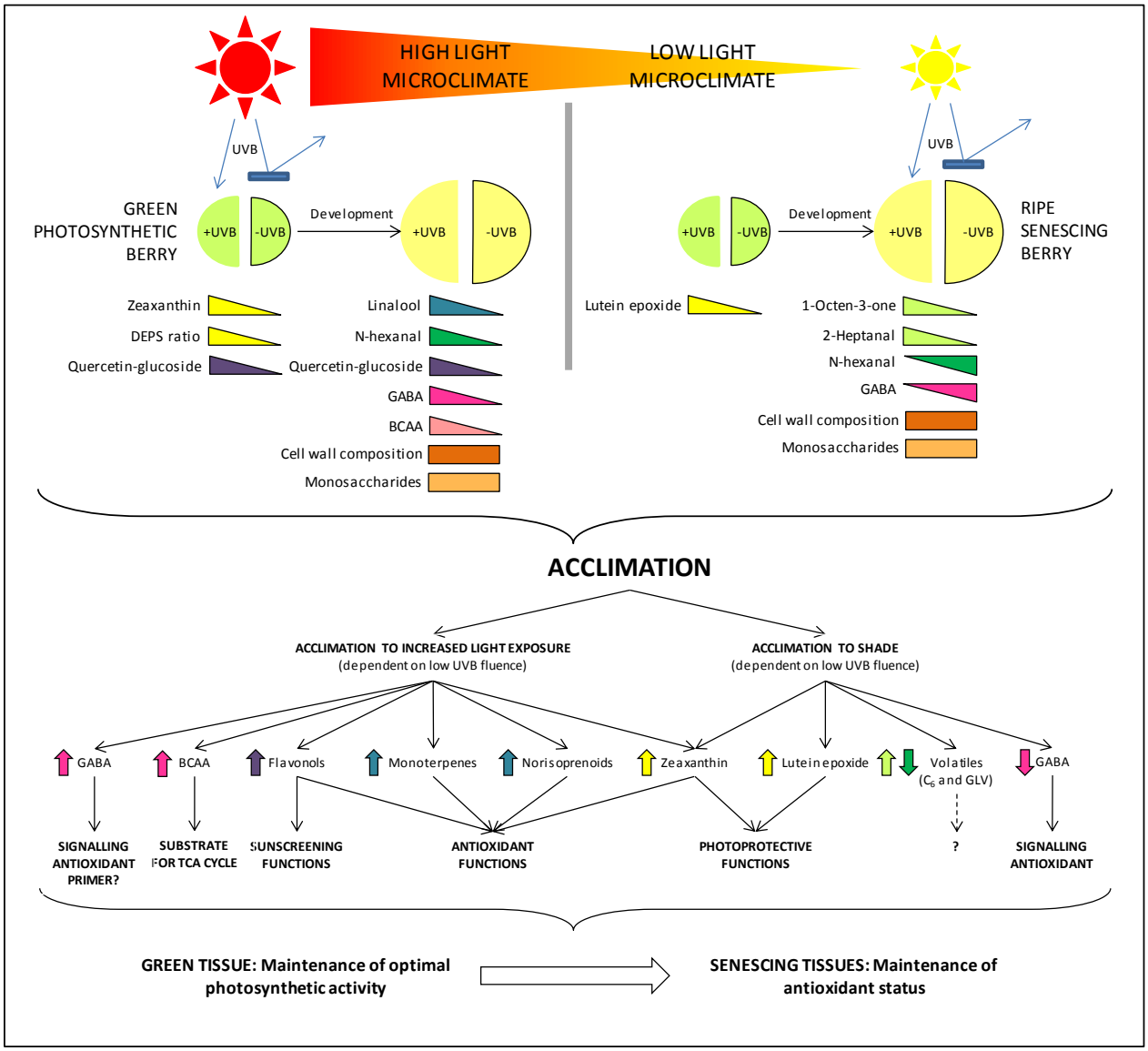


Figure 4.4. A comprehensive model summarising the early and late developmental stage metabolic responses in grape berries as a way to acclimate to variations in UVB light under HL and LL microclimates. In each light environment (HL and LL) both early and late developmental stages are represented as well as the attenuation of UVB. The coloured triangles indicate those compounds that reacted to UVB attenuation in each case, indicating the presence of an acclimation response in the berries. Each of the compound groups perform a specific function in the berry tissue and contribute to the acclimation of the berry via various physiological processes. These processes differ depending on the tissue type and are therefore associated with the developmental stage of the berry.

4.4 Discussion

The impact of the four microclimates established in this study on the compositional changes to the berry tissue cell walls, as well as the responses of the berry amino acids were evaluated. A recently proposed theoretical berry cell wall model (Gao et al., 2016a) served as a basis to compare our profiling of the cell walls from the skin and pulp tissues. This model was based on the analysis of grape berry pomace (*Vitis vinifera* cv. Cabernet Sauvignon) using the techniques outlined in this study. Interestingly, the confirmed variations to light quality and quantity in our experimental system did not lead to any significant changes in the cell wall composition of the skin or pulp cell walls of the ripe berries. Both the monosaccharide and polymer profiling analyses confirmed that only small, statistically insignificant changes were observed (Table 4.3 and 4.4). In other plant related studies, UVB radiation has been shown to elicit various responses in cell walls including physical modifications to cell wall elasticity (Lesniewska et al., 2004), higher peroxidase activity and lignin deposition in cell walls of epidermal cells (Hilal et al., 2007) and increased cell wall thickness (Álvarez-Gómeza et al., 2017). Although similar studies have not been done on ripe grape berries specifically, several studies have looked at the differences in cell wall composition between grape cultivars, while others have focused on the changes which occur during ripening and the potential influence of ripeness level at harvest (Gao et al., 2016b; Nunan et al., 1998; Ortega-Regules et al., 2008; Yakushiji et al., 2001). Our results suggest that the level of stress experienced in the treatments where light quantity and quality were modulated was not enough to physically alter the berry cell walls. The methods we used however did not investigate cell wall thickness and/or elasticity and it therefore cannot be ruled out that these aspects were not influenced by light exposure and/or UVB attenuation.

The berry amino acid profiling results, however, confirmed significant changes to the amino acid profile and levels under the different microclimates established in this study. The primary and secondary metabolite profiling (presented in Chapter 3) was extended in this chapter to include the analysis of 22 amino acids throughout berry development in the four distinct microclimates. Overall, the data confirmed developmental patterns as a strong driver in the responses of the berries to the four microclimates. Moreover, the level of light exposure also influenced the responses of the amino acid levels and profiles to UVB attenuation.

A number of important amino acids were seen to increase during berry development and ripening, however a greater pool of total amino acids was seen in the green developmental stage; this can be ascribed to the high levels of glutamine at this stage, it being the most abundant amino acid (Glad et al., 1992; Gourieroux et al., 2016). Amino acids are integral to development of berry tissues and generally increase in the berry during growth and ripening. Although amino acid synthesis occurs in grape berries, the majority of amino acids are imported from other plant organs via the xylem and phloem tissues. The

amino acid profile in translocating tissues will differ according to cultivar, but the most abundant amino acids, namely glutamine, glutamate, and aspartate, are normally present along with others such as proline, alanine, arginine and glycine (Gholami et al., 2004; Glad et al., 1992; Gourieroux et al., 2016). As a main nitrogen transporter, it is possible that the observed high levels of glutamine in the green berries were broken down during development to release nitrogen, which could be assimilated to form other amino acids (Bernard and Habash, 2009; Gourieroux et al., 2016). The enzymes involved in glutamine synthesis and catabolism, namely glutamine synthase and glutamine dehydrogenase, as well as the action of incorporation of ammonia into amino acids has been demonstrated in grape berries.

Although developmental trends of amino acid accumulation were maintained, the variation in light exposure did trigger changes in amino acid levels at different stages (Table 4.2). Previous work in our research group has examined the effects of light exposure on amino acid metabolism (du Plessis et al., 2017). The authors showed that the photosynthesis related proteins were transcriptionally upregulated under HL conditions, indicating a potential increased need for them due to them continuously being broken down and replaced. The energy requirements for this acclimation strategy were said to come from amino acid catabolism, which provided precursors and substrates to be used in stress mitigation mechanisms (du Plessis et al., 2017). There is a distinction to be made between "levels and turnover." A level can be maintained, regardless of whether the proteins are maintained or if they are being broken down and then replaced. The study by du Plessis et al. (2017) showed that HL conditions lead to lowered amino acids in the berries, similarly to what was seen in this study. HL conditions also upregulated transcription for photosynthesis-related proteins, which was used to explain the lowered levels of amino acids under these conditions due to them being incorporated into these proteins.

Within these light environments (HL and LL), the results of the study presented in this chapter showed that amino acids only responded significantly to variations in UVB radiation in the late developmental stage with the most significant responses involved in GABA and the branched chain amino acids (valine, leucine and isoleucine) levels (Figure 4.3). In the HL microclimate, GABA was increased in the ripe berries, but UVB attenuation led to significantly lowered levels. Converse results were seen in the LL microclimate; GABA levels were higher with an attenuation of UVB and the GABA content in the HL-UVB and LL-UVB berries were similar. Furthermore, the branched chain amino acids BCAA were shown to be higher in the HLcontrol environment, while no UVB attenuation responses were seen in the LL microclimate.

Berry acclimation to UVB radiation has been shown to involve various metabolites (Chapter 3) and this working hypothesis was extended to include the amino acids that responded significantly to the conditions created in the four microclimates. Potential roles of the different responsive amino acids in berry acclimation are included in the proposed model (Figure 4.4). Possible roles include the

involvement of amino acids in the maintenance of antioxidant homeostasis, possible priming of berries for efficient and effective implementation of stress mitigation strategies and the possible use of amino acid degradation products as an energy source for respiration and stress responses as is discussed below.

A number of studies have demonstrated the relationship between UVB radiation exposure and the consequent increase in GABA. Numerous papers and reviews have been published outlining the role of GABA as a metabolite and its involvement in stress tolerance and adaption (AL-Quraan, 2015; Bouché et al., 2003; Bouché and Fromm, 2004; Fait et al., 2005; Shelp et al., 1999). Furthermore, the signalling role of GABA in stress mitigation has long been speculated and research into the possibility has been conducted over the last two decades. A comprehensive review has since been published on GABA signalling in plants (Ramesh et al., 2017). This amino acid, acting either as a metabolite or a signalling molecule, has therefore been implicated in abating oxidative damage by restricting the accumulation of ROS and in maintaining the antioxidant homeostasis of plant cells. Considering the role of GABA, it is possible that this compound accumulated in grape berries as a way to deal with UVB radiation and thereby avoid damage. The previous chapter discussed the involvement of various metabolites in berry acclimation to UVB radiation and it is possible that GABA may also play a role in this, suggesting that the berry employs various mechanisms to prevent UVB induced damage and thereby remains healthy.

Furthermore, it could be speculated that GABA acts as a primer for stress in the HLcontrol berries. GABA priming has been shown to reduce the rate of ROS induced lipid peroxidation in black pepper plants during osmotic stress (Vijayakumari and Puthur, 2016). This study demonstrated that the pre-treatment of black pepper plants with GABA induced improved defence responses to osmotic stress. It was shown that GABA priming stimulated the endogenous synthesis of GABA upon exposure to osmotic stress, thereby allowing the plant to react swiftly and more efficiently to counteract this stress, utilising the inherent metabolic and signalling functions of GABA in abiotic stress mitigation. It is therefore possible that in field conditions in the HLcontrol microclimate, increased GABA accumulation during ripening may impart a priming effect to enable the berry to respond more effectively to UVB stress.

In the LL microclimate GABA was maintained at a lower level and an attenuation of UVB lead to an increase in GABA. These berries, being the least acclimated to both light and UVB would be stress-prone due to the absence of UVB and therefore susceptible to oxidative damage upon exposure. This hypothesis is also supported by the variations seen in the C6 compounds and GLVs under HL and LL conditions as described in Chapter 3. Light conditions within a canopy are not stable and sudden bursts of light may reach these berries. Due to their lack of other protective compounds such as phenolic compounds or monoterpenes (as discussed in chapter 3), it might be possible that GABA accumulation

was stimulated in these berries as a way to deal with this sudden stress and thereby prevent oxidative damage .

Furthermore, although the mechanisms involved with increased BCAA levels have not been extensively studied, some literature provides an interesting context that could form part of future work. The degradation products of branched chain amino acids can be fed into the TCA cycle and can therefore act as alternate substrates for respiration. The electron-transfer flavoprotein:ubiquinone oxidoreductase (ETF/ETFQO) is a nuclear-encoded protein situated in the inner mitochondrial membrane and has been identified in plants (Heazlewood et al., 2004). EFT is able to accept electrons from various mitochondrial matrix flavoprotein dehydrogenases and transfer them to ubiquinone, thereby catalysing the reduction of ubiquinone by EFT in the inner mitochondrial membrane. The complex has been shown to be associated with the catabolism of certain amino acids, with the degradation products of BCAAs specifically acting as substrates in the ETF/ETFQO pathway and serving as alternative electron donors in the electron transport chain (Araújo et al., 2010; Ishizaki et al., 2005; Zhang et al., 2006). The pathway has been shown to be induced by various external stimuli, including dark induced senescence, water restrictions and oxidative stress conditions (Buchanan-Wollaston et al., 2005; Lehmann et al., 2009; Pires et al., 2016). Our results were consistent with this interpretation, namely that under conditions of increased UVB exposure and a consequent increase in the potential for oxidative stress, these amino acids accumulate to "feed" the process of respiration, which may be induced under these conditions. It can be furthermore speculated that the metabolism of BCAA could occur to provide carbon skeletons to be used in the synthesis of certain compounds which accumulate under UVB, leading to an increased accumulation of them under these conditions.

The propensity for stress under the different UVB exposures has stimulated the evolution of various acclimation strategies including the accumulation of antioxidant volatiles and polyphenolic compounds (Chapter 3). The results of this study demonstrated that GABA itself may be implicated in mitigating stress responses and maintaining the antioxidant homeostasis in berries through their metabolic and signalling actions. It was already previously suggested that GABA accumulation in the berry may serve as a primer, which could enable the berry to respond more efficiently and effectively to UVB stress. It is furthermore possible that the energy requirements of these processes is subsidised by the branched chain amino acids. These results therefore provide further insights into the capacity of berries to acclimate to UVB exposure and thereby mitigate any potential damage.

It is however important to note that taking into consideration the seasonal variability occurring in field conditions, definitive conclusions cannot be made with certainty in those situations where differences were only measured in one season. The results do however provide interesting insights into berry responses and future work should consider multiple seasons to extend these results. However, the

previous trial conducted in the experimental vineyard on amino acid responses to light variability by du Plessis et al. (2017) showed interesting responses, further substantiating the influence of light on amino acids. Here it was shown that genes encoding for amino acid catabolism were more upregulated in the exposed treatment and this break down of amino acids lead to the production of substrates which could be used in energy costly stress defence mechanisms. Furthermore, it should be noted that amino acid analysis was conducted on whole berries and considering the compartmentalisation of amino acids to certain berry tissues, it would be interesting to consider tissue specific accumulation patterns in future studies.

4.5 References

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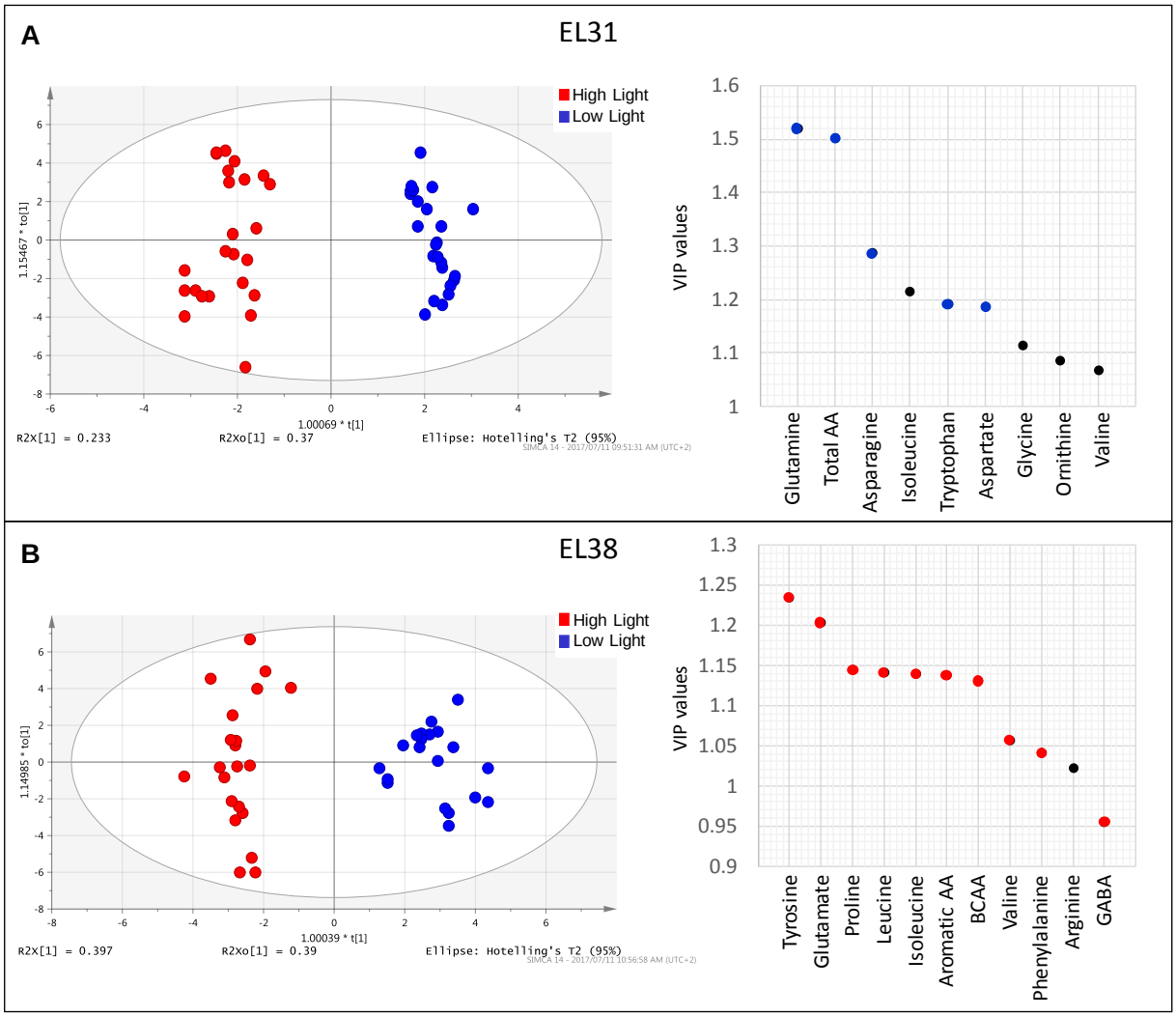
Supplementary data to Chapter 4

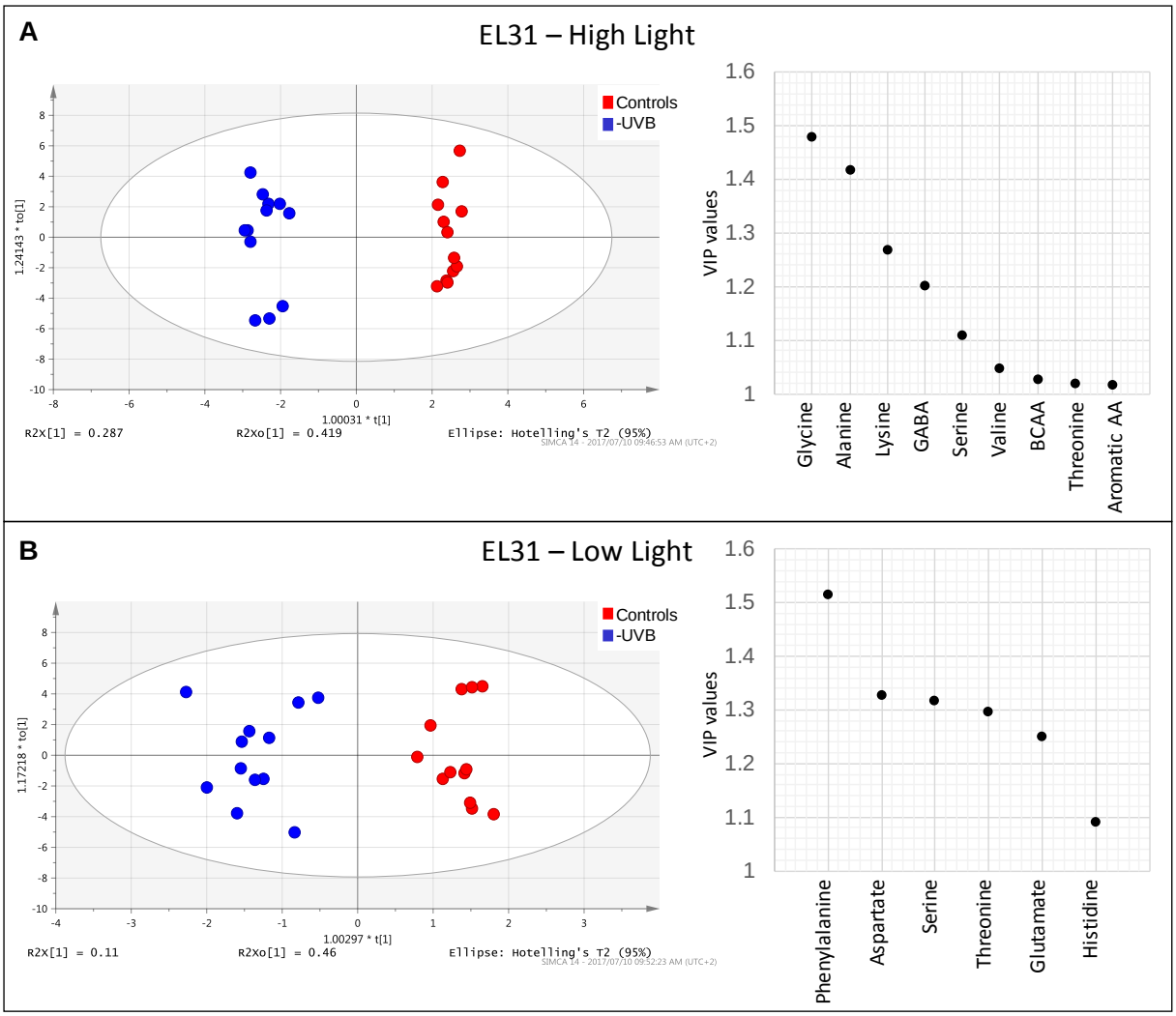
Supplementary table 4.1 A table listing the measured contents of all the compounds ± SD. The log_2-fold changes and corresponding p-values between the HL control/ HL-UVB and LL control)/LL-UVB contrasts are calculated and listed for each compound at each developmental stage.

Compound	EL31: HLcontrol	EL31: HL-UVB	Fold change (log2)	p-value	EL31: LLcontrol	EL31: LL-UVB	Fold change (log2)	p-value
Amino acids (2014/2015)								
Aspartate (Asp)	21.7±5.3	22.8±6.5	-0.07	0.64	35.7±4.1	29±8.3	0.30	0.02
Glutamate (Glu)	151.6±30	191.1±35.9	-0.33	0.01	182.6±13.6	162.3±25.5	0.17	0.02
Cysteine (Cys)	15.7±3.6	18±5.3	-0.20	0.03	18.5±4.9	17.4±3.7	0.09	0.54
Asparagine (Asn)	28.7±7.7	31.1±6.5	-0.12	0.41	53.7±11.9	46.4±12.8	0.21	0.16
Serine (Ser)	55.4±8.3	69.6±9.8	-0.33	0.00	64.5±7.7	53.5±12.1	0.27	0.01
Glutamine (Gln)	2400.4±432.4	2697±692.6	-0.17	0.22	4941±916.7	4864±544	0.02	0.80
Histidine (His)	10.3±3	10.1±2.1	0.02	0.87	11.8±1.1	11.6±2.6	0.02	0.12
Glycine (Gly)	0±0	11.8±2.2		0.00	0±0	0±0		
Threonine (Thr)	16±2.6	19.8±3.6	-0.31	0.01	19.5±2.5	17±2.4	0.20	0.02
Arginine (Arg)	0±0	0±0			0±0	0±0		
Alanine (Ala)	13.6±1.9	21.7±1.7	-0.67	0.00	19.3±1.9	17.6±2	0.13	0.05
Tyrosine (Tyr)	6±1.5	7.1±1.3	-0.24	0.08	5.4±0.8	5.4±1	0.02	0.83
Cys-cys (Cys-Cys)	4.9±4.6	4.4±1.6	0.15	0.74	3.9±0.7	3.2±0.5	0.27	0.05
Valine (Val)	6.9±0.8	8.3±5.2	-0.27	0.00	10±1.2	9.9±3.9	0.01	0.96
Methionine (Met)	0±0	0±0			0±0	0±0		
Tryptophan (Trp)	8.5±1.3	8.7±1.5	-0.04	0.67	10.4±1.2	10.3±1.1	0.01	0.89
Phenylalanine (Phe)	5.3±1.4	6.3±0.9	-0.25	0.05	6.9±1	5.5±0.9	0.33	0.00
Isoleucine (Ile)	5.2±0.7	3.2±0.7	0.68	0.01	0±0	0±0		
Ornithine (Orn)	5±1	6.6±1.1	-0.38	0.00	6.3±1.7	4.3±0.3	0.56	0.16
Leucine (Leu)	4.9±0.8	2.9±0.2	0.75	0.77	0±0	0±0		
Lysine (Lys)	0±0	2.9±1.8		0.00	0±0	3.9±0.4		0.07
Proline (Pro)	0±0	0±0			0±0	0±0		
GABA	16.64±4.49	29.48±7.83	-0.83	0.00	20.13±1.08	20.12±5.66	0.00	1.00
Aromatic AA	21.49±6.65	30.4±7.77	-0.50	0.01	32.69±3.51	31.07±6.17	0.07	0.44
Branched chain amino acids	4.23±7.71	12.96±5.17	-1.62	0.00	9.97±1.2	9.91±3.87	0.01	0.96
Total amino acids	2759.92±491.58	3171.39±748.26	-0.20	0.13	5401.55±965.05	5275.71±576.32	0.03	0.70
Compound	**EL35: HLcontrol**	**EL35: HL-UVB**	**Fold change (log2)**	**p-value**	**EL35: LLcontrol**	**EL35: LL-UVB**	**Fold change (log2)**	**p-value**
Amino acids (2014/2015)								
Aspartate (Asp)	41.8±4	42.8±4.9	-0.03	0.58	47.6±7.7	56.1±9.7	-0.24	0.03
Glutamate (Glu)	242.5±18	252.4±27.4	-0.06	0.30	249.7±34.4	281.8±47.4	-0.17	0.07
Cysteine (Cys)	0±0	0±0			10.4±1.3	9.8±1.3	0.09	0.04
Asparagine (Asn)	0±0	0±0			20.4±2.6	21.7±3.7	-0.09	0.19
Serine (Ser)	43.9±4.9	45.7±5.7	-0.06	0.43	40.4±7.4	48.8±8.1	-0.28	0.01
Glutamine (Gln)	976.3±330.6	881.9±250.7	0.15	0.44	1647.2±180.8	1586.4±451.9	0.05	0.67
Histidine (His)	19.8±3.4	17.8±3.6	0.15	0.18	18.9±4.1	21.7±4.4	-0.20	0.12
Glycine (Gly)	0±0	0±0			0±0	0±0		
Threonine (Thr)	54.6±5.2	55±8.6	-0.01	0.88	41.4±4.6	49.1±6.8	-0.24	0.00
Arginine (Arg)	290.8±41.6	277.4±60.3	0.07	0.53	314.7±55.7	381.9±85.2	-0.28	0.03
Alanine (Ala)	56.5±5.8	51.6±9.6	0.13	0.14	63±11.5	75.6±16	-0.26	0.04
Tyrosine (Tyr)	10±2.9	9.8±2.1	0.02	0.87	11.7±0.9	12.6±2.9	-0.11	0.32

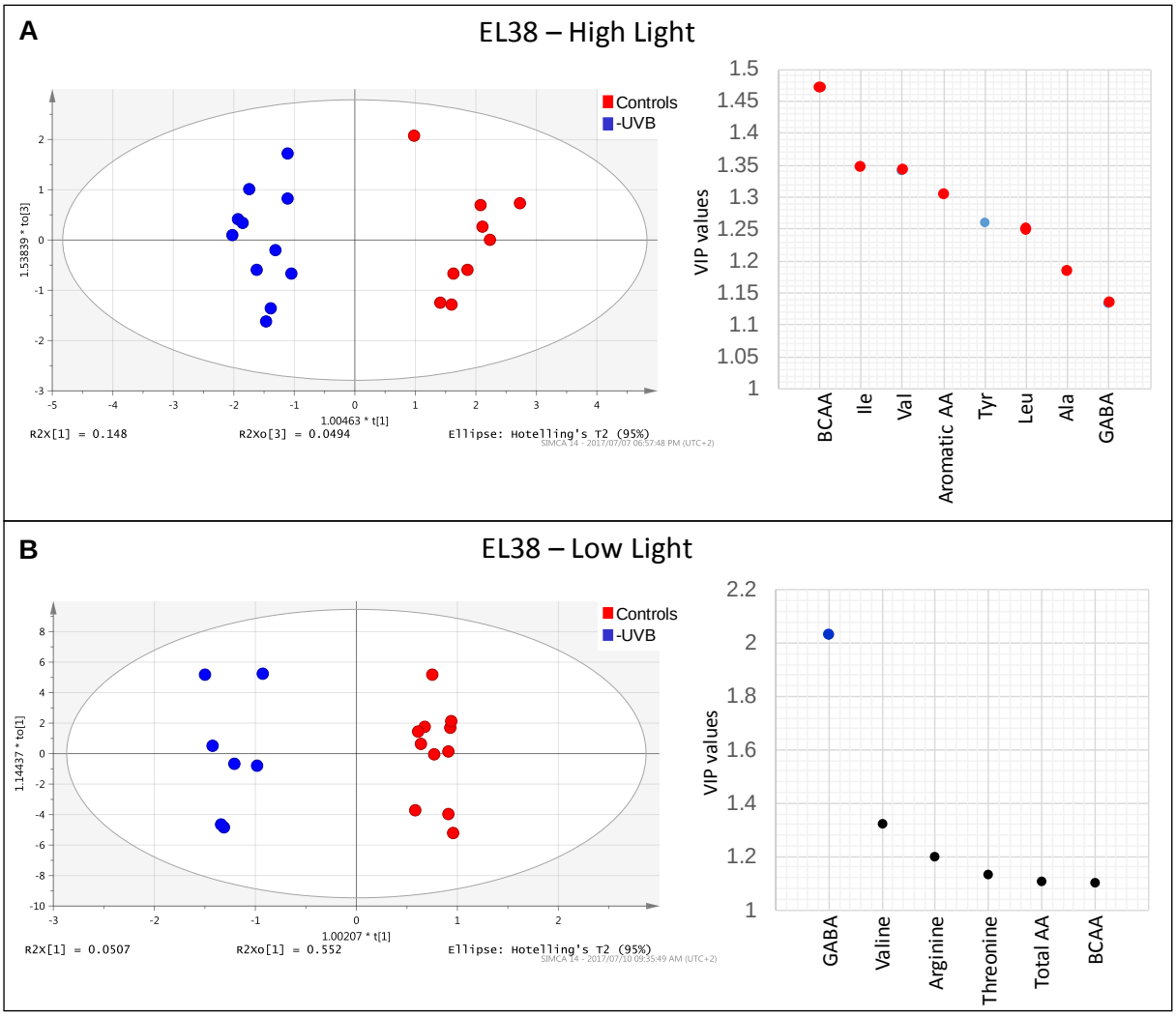
Cys-cys (Cys-Cys)	3.8±1.2	4.2±0.3	-0.15	0.03	3.3±0.9	4.2±0.8	-0.37	0.01
Valine (Val)	10.6±1.9	9.9±3	0.09	0.53	7.5±2	9.9±2.8	-0.41	0.02
Methionine (Met)	0±0	0±0			0±0	0±0		
Tryptophan (Trp)	5.2±1.8	4.4±0.9	0.22	0.21	6.2±0.5	7.6±1.7	-0.31	0.01
Phenylalanine (Phe)	4.8±1.7	4.6±1.2	0.06	0.74	4.1±0.8	5.2±1	-0.33	0.01
Isoleucine (Ile)	6.4±0.7	5.7±1.7	0.18	0.16	5±1	5.7±1	-0.17	0.14
Ornithine (Orn)	9.2±1.3	7.5±1	0.30	0.00	8.1±1.4	7.8±1.7	0.06	0.63
Leucine (Leu)	4.4±0.6	4.2±1.5	0.06	0.71	4.1±1.2	4.3±1.1	-0.08	0.64
Lysine (Lys)	4.7±0.4	4.5±0.8	0.09	0.00	5.1±1.2	5±1.6	0.03	0.87
Proline (Pro)	60±13.1	72.4±23.4	-0.27	0.63	0±0	53.9±14.1		0.00
GABA	60.43±7.11	53.98±6.16	0.16	0.03	49.49±8.99	68.65±13.74	-0.47	0.00
Aromatic AA	30.45±6.03	28.7±6.65	0.09	0.51	29.48±3.39	35.33±8.26	-0.26	0.03
Branched chain amino acids	21.39±2.47	19.8±6.09	0.11	0.41	16.58±3.8	19.88±4.78	-0.26	0.07
Total amino acids	1904.69±366.02	1775.46±304.01	0.10	0.36	2558.35±222.64	2696.55±636.92	-0.08	0.49
Compound	**EL38: HLcontrol**	**EL38: HL-UVB**	**Fold change (log2)**	**p-value**	**EL38: LLcontrol**	**EL38: LL-UVB**	**Fold change (log2)**	**p-value**
Amino acids (2014/2015)								
Aspartate (Asp)	42.2±18.2	37.8±10.9	0.16	0.48	62±15.1	51.3±7.8	0.27	0.04
Glutamate (Glu)	448.9±39.8	425.3±44.7	0.08	0.19	312.7±26.1	352.6±71.8	-0.17	0.08
Cysteine (Cys)	0±0	0±0			0±0	0±0		
Asparagine (Asn)	0±0	0±0			0±0	0±0		
Serine (Ser)	47.6±6.1	46.7±8.2	0.03	0.75	42.6±8.3	44.4±8.5	-0.06	0.61
Glutamine (Gln)	424.3±181	397.8±152.1	0.09	0.70	543.3±79.4	609.8±111.8	-0.17	0.11
Histidine (His)	33.4±6.3	28.2±10.2	0.24	0.15	24.2±5.6	28±7.4	-0.21	0.17
Glycine (Gly)	0±0	0±0			0±0	0±0		
Threonine (Thr)	76.6±9.3	72.2±12.9	0.08	0.35	63.4±8.1	72.2±11.6	-0.19	0.04
Arginine (Arg)	631.7±167.8	541.7±165	0.22	0.20	779.6±72	770.1±147.7	0.02	0.84
Alanine (Ala)	134.3±15.8	112.3±22	0.26	0.01	106.2±25.5	110.8±21.5	-0.06	0.64
Tyrosine (Tyr)	18.4±1.3	15.8±3.2	0.22	0.02	9.3±1.4	10.4±3.2	-0.16	0.28
Cys-cys (Cys-Cys)	2.6±0.3	4.1±0.8	-0.64	0.00	4±1.2	3.7±1.5	0.12	0.57
Valine (Val)	42.1±9.6	28.9±10.6	0.54	0.00	16.2±2	22.4±8.1	-0.47	0.02
Methionine (Met)	0±0	0±0			0±0	0±0		
Tryptophan (Trp)	4.1±0.7	3.7±1.1	0.14	0.30	3.4±0.8	3.7±0.8	-0.12	0.35
Phenylalanine (Phe)	13.9±1.3	14.1±2.2	-0.02	0.82	11.1±1.4	12.5±2.2	-0.17	0.07
Isoleucine (Ile)	20.1±2.1	16.6±2.8	0.28	0.00	12.4±1.2	12.6±2.9	-0.02	0.88
Ornithine (Orn)	8±1.5	7.3±1.9	0.14	0.32	6.1±0.7	6.9±1.7	-0.19	0.13
Leucine (Leu)	23.2±1.8	19.3±3.5	0.26	0.00	14.6±1.7	16.6±4	-0.19	0.12
Lysine (Lys)	9±1.5	8.3±2.7	0.12	0.42	7.1±0.7	7.6±1.8	-0.11	0.33
Proline (Pro)	324.4±35.5	323.8±53.3	0.00	0.97	197±26.2	222.4±82.4	-0.18	0.32
GABA	69.03±18.78	55.85±12.98	0.31	0.06	40.77±7.68	55.35±8.63	-0.44	0.00
Aromatic AA	78.56±10.73	62.57±15.43	0.33	0.01	39.86±4.89	48.92±13.03	-0.30	0.03
Branched chain amino acids	85.44±11.08	64.8±14.58	0.40	0.00	43.2±4.74	51.58±12.46	-0.26	0.04
Total amino acids	2373.4±379.57	2159.85±459.52	0.14	0.23	2255.89±206.3	2413.23±151.33	-0.10	0.04



Supplementary figure 4.1 OPLS-DA models generated for the amino acid data using light exposure as the y-variable for both the early stage (A) and late stage (B). Each OPLS-DA is accompanied by a plot of compounds which contributed most to the model according to the VIP list. Those amino acids indicated with a blue dot were significantly higher in the LL microclimate and the compounds indicated with a red dot are those which are significantly higher in the HL microclimate. The black dots represent those amino acids which did not respond significantly. Significance was determined with a Fisher LSD post hoc test.



Supplementary figure 4.2 OPLS-DA models generated for the amino acid data using light UVB attenuation as the y-variable for both the high light (A) and low light (B) microclimates in the early developmental stage. Each OPLS-DA is accompanied by a plot of compounds which contributed most to the model according to the VIP list. The black dots represent those amino acids which did not respond significantly. Significance was determined with a Fisher LSD post hoc test and no amino acids were highlighted as significant in this developmental stage.



Supplementary figure 4.3. OPLS-DA models generated for the amino acid data using light UVB attenuation as the y-variable for both the high light (A) and low light (B) microclimates in the late developmental stage. Each OPLS-DA is accompanied by a plot of compounds which contributed most to the model according to the VIP list. Those amino acids indicated with a blue dot were significantly higher in the LL microclimate and the compounds indicated with a red dot are those which are significantly higher in the HL microclimate. The black dots represent those amino acids which did not respond significantly. Significance was determined with a Fisher LSD post hoc test.

Chapter 5

A comparison of Sauvignon Blanc juice composition, analysed at three juice-processing steps to evaluate the impacts of UVB attenuation in high and low light microclimates

5.1 Introduction

In white wine making, ripe grape berries are harvested and subjected to a number of different processing techniques, each of which can influence the juice matrix characteristics. The resulting juices comprise numerous and varied compounds including sugars, acids, phenolic and grape-derived aroma compounds and precursors and is considered the fermentation matrix/medium. The juice matrix is typically analysed with basic analyses and certain parameters are considered before additions to this matrix are made to optimally support the subsequent wine fermentation. The context of the grapes during the growing season and the final composition of the berries on the harvest date define the potential of the juice (Chambers and Pretorius, 2010; Mills et al., 2008; Swiegers et al., 2005).

The processing of the berries to obtain the juice elicits various changes, as does the handling of the juice once acquired (Coetzee and du Toit, 2012). Berry processing and juice preparation may include procedures such as crushing, pressing, fining, filtration and exposure to skin contact. Various studies have investigated how these processes can influence berry juice composition. During crushing for example, phenolic compounds such as flavonoids are released from the skin and seeds into the juice matrix. Terpenes may also be liberated from sugar molecules during crushing due to a resultant increase in the activity of certain enzymes such as β-glucosidase (Cordonnier and Bayonove, 1981). Different pressing pressures have been shown to influence the varietal thiols in the wine, glutathione content and oxidation potential (Ferreira-Lima et al., 2016; Maggu et al., 2007; Patel et al., 2010). It has also been demonstrated that the use of fining agents prior to fermentation may potentially affect certain aromatic compounds found in the wine (Parish et al., 2016).

Skin contact, in particular, could have a profound influence on juice and wine properties, with most trials focussing on red varietals. Studies in white cultivars however have shown an increased extraction of certain varietal compounds such as 3-mercaptohexan-1-ol precursor (P-3MH), a decline in glutathione and an increase in polyphenolic compounds (Gawel et al., 2014; Gómez-Míguez et al., 2007; Maggu et al., 2007; Peyrot-des-Gachons et al., 2002). Amino acids which can directly impact fermentation and the resulting production of certain aroma compounds (Styger et al., 2011) are also extracted from the berry. The localisation of the amino acids in the grape berry remains somewhat unclear due to conflicting results, however the most recent evidence suggests that they also accumulate

mostly in the skin (Guan et al. 2017). Furthermore, the polyphenolic compounds that contribute significantly to the organoleptic properties of the wines by modifying mouthfeel and taste, are generally found in the berry skin and seed tissues and can be extracted during juice processing. In commercial winemaking, any, or all of these procedures may be employed, essentially modulating the transfer of the metabolic composition that was originally found in the harvested berry, to the subsequent juice and fermenting must media.

Sauvignon Blanc wines are made across the wine-making areas of the world and the grapes are normally harvested between 19 and 21 degrees balling (Hunter et al., 2004; Marais, 1998). The juice and wine composition is influenced by several factors. For example, mechanical harvesting has been shown to influence the aroma composition of wine by increasing varietal thiols and certain C_6 alcohols (Herbst-Johnstone et al., 2013). Tian et al. (2013) furthermore showed that mechanical harvesting lowered protein levels in juice and wine when compared to hand harvesting. Research has also been conducted on a number of pre-fermentation treatments and fermentation conditions to determine their effects on Sauvignon Blanc juice and wine characteristics. For example, during winemaking, juice contact with excessive oxygen can lead to oxidation, juice browning and the loss of aroma compounds (Patel et al., 2010). Sauvignon Blanc grapes are therefore typically processed under reductive conditions by utilising inert gasses like nitrogen or inert presses to displace oxygen and thereby reduce oxidation (Coetzee and du Toit, 2012). Temperature also plays a significant role in influencing juice and wine composition, with different timing eliciting variable results. Higher grape storage temperatures have been shown to increase monoterpene extraction into the juice, reduce esters and increase polyphenols prior to fermentation (Marais, 1998), while elevated temperatures during pressing may lead to an increased incidence of thiol precursors in the juice and manipulation of fermentation temperatures will influence the development of thiols from these precursors (Masneuf-Pomarède et al., 2006). Pressing pressures may also significantly alter Sauvignon Blanc juice composition. Higher pressures have been shown to increase the extraction of polyphenolic compounds in terms of for example, the content of thiol precursors and polyphenolic compounds (Maggu et al., 2007; Patel et al., 2010). Furthermore, skin contact, either as a stand-alone treatment, or in conjunction other conditions such as temperature and pressing pressure, will yield significant differences in juice composition. Typically, skin contact will increase the content of polyphenolics in the juice, but may also increase the presence of thiol precursors, which may be exacerbated by higher pressures and temperatures. Slight increases in methoxypyrazines have also been noted with skin contact (Maggu et al., 2007), while monoterpene extraction could be significantly enhanced (Marais, 1998). The review by Coetzee and du Toit. (2012) provides further information on the effects of these different processes on Sauvignon Blanc juices and wines.

Further Sauvignon Blanc wine variability may be elicited by choice of yeast strain for fermentation. Different strains differ in their ability to form various odour related compounds such as thiols and esters.

For example, VIN7 and VIN13 (Anchor Yeast Biotechnologies) are commonly used as they have a high affinity for releasing volatile thiols from their related precursors, although each yeast more strongly produces different thiols. Research conducted at the Australian Wine Research Institute by Swiegers et al. (2006) provided an excellent summary of different yeast characteristics and their influence on Sauvignon Blanc.

Different additions prior to fermentation will also influence Sauvignon Blanc juice and wine characteristics. Most commonly used in winemaking practices is the addition of SO_2 as an antioxidant. Coetzee (2011) investigated the effects of SO_2 additions to Sauvignon Blanc and found a positive influence on wine volatile thiols as they were protected from oxidation. Certain esters were also found to be higher in content with the addition of SO_2. Another common practice in winemaking is the addition of diammonium phosphate (DAP) to the juice prior to fermentation to supplement nitrogen needed by the yeast. This practice significantly alters aroma compound composition, affecting specifically the fermentation derived volatile compounds. In general, addition of DAP will reduce the content of higher alcohols in the wine, but will elevate both ethyl and acetate esters (Ugliano et al., 2007). In Sauvignon Blanc, DAP additions have been shown to increase the content of certain volatile thiols, namely 3MHA and also affected ester formation (Pinu et al., 2014).

Given the strong and well documented characterisation of Sauvignon Blanc wine styles with information on the influence of varietal grape characteristics, it is an excellent cultivar to evaluate the transitions in compounds from grapes to juice to wine. The approach in this study was to generate and confirm microclimatic conditions in a Sauvignon Blanc vineyard where the impacts of UVB in both a high light (HL) and low light (LL) environment on berry metabolism were studied (refer to Chapters 3 and 4). Here the post-harvest, but pre-fermentation stages of juice preparation were targeted for evaluation. The grapes from the four microclimates were harvested and subjected to steps to generate juices. The metabolite profiling conducted to characterise the grapes were also applied to the juice matrices and three processing steps, before the inoculation of the yeast and onset of alcoholic fermentation, were evaluated. The results showed significant compositional shifts between the ripe grape berry and juice samples in terms of compound concentrations and/or profiles. Furthermore, variations in UVB radiation led to interesting responses in the juice components and the degree of light exposure was seen to strongly modulate the UVB responses.

5.2 Materials and Methods

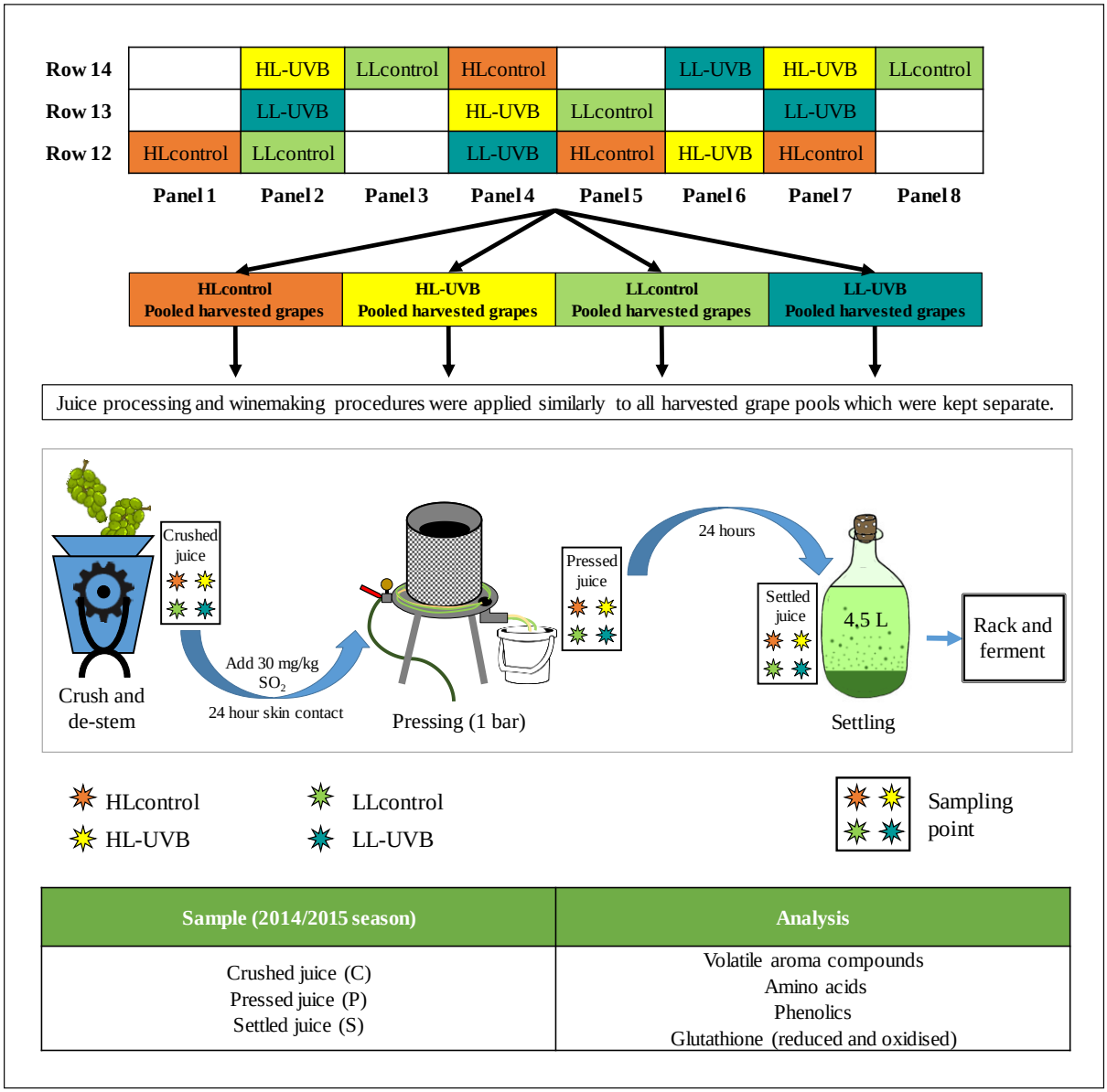
5.2.1 Vineyard treatment and juice sampling

Different microclimates were created in the vineyard using canopy management and the inclusion of UVB attenuating Perspex sheets. This experimental setup was validated and is presented in Chapter 3. The microclimates included those generated by a HLcontrol, HL-UVB, LLcontrol and LL-UVB treatment and the experimental layout and steps involved for the juice analysis (in context of the wine-making process is shown in Figure 5.1). The harvesting of the grapes occurred in conjunction with the commercial harvest for that specific vineyard block. The two controls and two treatments were each harvested separately at around 21 to 22°B on the same day. This was decided based on the fact that over multiple seasons, the impact of development and ripening on sugar levels was marginal, particularly with UVB attenuation in both the HL and LL microclimates (Figure 5.1). Particular attention was paid with regards to which bunches were selected for harvesting. Only sun-exposed bunches were chosen for the HLcontrol and HL-UVB, while only shaded bunches were selected for the LLcontrol and LL-UVB treatments. A rough estimate of the average berry fresh mass was also determined in each season (after harvesting of the grapes) by weighing 20 randomly selected berries per treatment together and dividing that number by 20.

Small-scale experimental wine was made from these treatments for two experimental seasons (2013/2014 & 2014/2015) according to the following pre-fermentation schedule: Grapes were pooled from the four biological vineyard repeats per microclimate, crushed and then stored overnight at 4°C. During crushing, SO_2 was added as well as dry ice to prevent oxidation. Following a 24 hour cold maceration period, the grapes were pressed in a hydraulic press at 1 bar pressing pressure, again using dry ice to prevent oxidation of the juice. The juices were allowed to clarify overnight at 4°C before continuing with the alcoholic fermentation and wine analyses (presented in Chapter 6). Moreover, all processes were conducted under reductive conditions using dry ice and CO_2 gas to displace oxygen.

In terms of the analysing the juice components during the two seasons, in the first season only conventional juice analysis was done of the juice obtained directly after crushing and included measuring °Brix, titratable acidity (TA) and pH. These were single measurements conducted on one sample from each treatment. In the second season (2014/2015), in addition to the conventional analyses, a more detailed analysis was conducted, including three distinct juice processing steps, as well as additional analyses for targeted metabolite compounds such as glutathione, grape-derived volatile compounds, polyphenolics and amino acids (an overview is presented in Figure 5.1). The three juice-processing steps were used as sampling points, generating crushed juice (C), pressed juice (P) and settled juice (S) samples. Three technical repeats were included for each of the four treatments. The juices were mixed up before sampling into 50 ml falcon tubes, barring the settle juice sample which

was taken carefully from the clear juice above the sediment. All samples were frozen immediately in liquid nitrogen and stored at -80°C until analysis.



Sample (2014/2015 season)	Analysis
Crushed juice (C) Pressed juice (P) Settled juice (S)	Volatile aroma compounds Amino acids Phenolics Glutathione (reduced and oxidised)

Figure 5.1. The juice processing procedure employed during the experiment for the two seasons. Juice sampling points are indicated on the figure. Each of the coloured stars represent a different microclimate. The table indicates the extra analyses conducted in the second season for the three different juices (3 technical repeats included).

5.2.2 Chemical analysis of juice samples

The juice samples were thawed at 4°C and different analyses were conducted simultaneously to evaluate their metabolic composition. Different methods were employed to analyse the different metabolites and these are described below.

5.2.2.1 Analysis of juice glutathione

The juice samples were prepared and analysed according to the method described in (Kritzinger et al., 2013). Analysis was conducted at the LC-MS laboratory at the Central Analytical Facility. The data was normalised and expressed in mg/L.

5.2.2.2 Analysis of juice volatile compounds

The volatile compounds were extracted from grape juice samples using head-space solid-phase-micro-extraction (HS-SPME). A juice sample or standard of 10 ml was transferred into a 20 ml Headspace vial and 50 µl of Anisole d8 as an internal standard was spiked into the solutions. Thereafter, 2.5 ml of 20% NaCl was added to the vials and vigorously vortexed before extraction with SPME fiber. This was done according to the method described in Chapter 3. Data was normalized and expressed in µg/L.

5.2.2.3 Analysis and quantification of amino acids and phenolic compounds

Juice samples of 1 mL were extracted and analysed for amino acids and phenolic compounds as is described in du Plessis et al. (2017). Data was normalized and expressed in mg/L.

5.2.3 Statistical analysis

Microsoft Excel and Statistica (version 12) were utilised for standard statistical analysis and the multivariate analysis was conducted using SIMCA (version 14 from Sartorius Stedim Data). To determine the effects of UVB, the metabolite data from all the microclimates was analysed on a "per processing stage" basis for each light exposure separately. The use of supervised OPLS-DA models assisted in the visualisation of the complex datasets which consisted of multiple variables and helped to identify putative correlations within the dataset. This data was further validated using factorial ANOVAs. Significance was tested using Fisher LSD Post Hoc tests to confirm which compounds reacted statistically significantly to the specified factors (adjusted p-value, q-value).

Furthermore, hierarchical clustering of metabolites was done to identify correlative patterns using Pearson correlation coefficients and confirm treatment responses in the different juice matrices. This was done using Expander (Developed at Ron Shamir's Computational Genomics group, Tel Aviv University, version 7.2). It is important to note that this type of cluster analysis relies on Pearson correlation coefficients to highlight compounds with similar trends, but does not give an indication of amplitude.

5.3 Results

5.3.1 Conventional juice analysis conducted over two seasons

The average berry fresh masses and conventional juice analyses per season are provided in Table 5.1. Although the berry fresh masses cannot be statistically justified due to single measurements, it was interesting to note the trend that berries from the HL environments displayed lower fresh masses compared to the LL environments over the two seasons. The Brix levels were always between 20°-22° Brix at harvest for both seasons and the other measured parameters were quite similar for the other parameters measured over the two seasons.

Table 5.1. Single measurements taken at grape harvest for berry fresh weight, Brix, titratable acidity and pH

	Date	Stage	Treatment	Berry FM (g/berry)	Brix	TA (g/L)	pH
Season 1	2014/03/06	EL38	HLcontrol	1.78	21.50	7.47	3.28
	2014/03/06	EL38	HL-UVB	1.92	22.00	6.31	3.39
	2014/03/06	EL38	LLcontrol	2.30	20.50	8.37	3.17
	2014/03/06	EL38	LL-UVB	2.40	20.00	8.84	3.16
Season 2	2015/02/20	EL38	HLcontrol	1.83	22.23	7.63	3.36
	2015/02/20	EL38	HL-UVB	1.86	22.23	7.87	3.30
	2015/02/20	EL38	LLcontrol	2.27	21.13	8.85	3.01
	2015/02/20	EL38	LL-UVB	2.20	21.20	9.02	2.98

The more detailed analysis of the different juice processing steps, conducted with FTIR analyses during the second season, is presented in Table 5.2.

Table 5.2. The Brix, total acid and pH measured in the different juices for the four microclimates by FTIR (n=3). Letters indicate significant differences per juice matrix.

Juice	Treatment	Brix	TA (g/l)	pH
Crushed juice	HLcontrol	22.4 ± 0.1^{a}	11.1 ± 0^{a}	3 ± 0^{a}
	HL-UVB	22.4 ± 0.1^{a}	11.4 ± 0.1^{a}	3 ± 0^{a}
	LLcontrol	21.2 ± 0.1^{b}	12.6 ± 0.2^{b}	3 ± 0^{a}
	LL-UVB	19.6 ± 0.1^{c}	12.9 ± 0.1^{b}	2.9 ± 0^{a}
Pressed juice	HLcontrol	22.5 ± 0^{a}	8 ± 0^{a}	3.2 ± 0^{a}
	HL-UVB	22.6 ± 0^{a}	8.3 ± 0^{a}	3.1 ± 0^{a}
	LLcontrol	21.5 ± 0^{b}	9.6 ± 0.1^{b}	3.1 ± 0^{a}
	LL-UVB	20.8 ± 0^{b}	9.9 ± 0^{b}	3.1 ± 0^{a}
Settled juice	HLcontrol	22.5 ± 0^{a}	8 ± 0^{a}	3.2 ± 0^{a}
	HL-UVB	22.6 ± 0^{a}	8.2 ± 0.1^{a}	3.2 ± 0^{a}
	LLcontrol	21.4 ± 0^{b}	9.6 ± 0.1^{b}	3.2 ± 0^{a}
	LL-UVB	20.8 ± 0^{b}	9.8 ± 0^{b}	3.1 ± 0^{a}

5.3.2 Chemical analysis of juice samples for amino acids, volatiles and phenolics

The data from all these different analyses is provided in Supplementary Table 5.1. It was clear that the different juice samples changed composition in terms of actual concentration of compounds measured as the juice processing steps proceeded.

Two approaches were taken to present the results; a more targeted approach to identify compounds responsive to the treatments summarised in Figure 5.2 (presented below per compound group in Figures 5.3, 5.4, 5.5 and 5.6), as well as hierarchical clustering (Figures 5.7, 5.8, 5.9 and 5.10) to group all compounds according to their similarity/dissimilarity in responses.

It was clear that light exposure remained a strong driver in the datasets. In order to identify metabolites responsive specifically to UVB in the C, P and S juice samples, each HL and LL juice was investigated separately using OPLS-DA models and ANOVA, validated by Fisher LSD post hoc tests and then summarised and presented in a Venn diagram (Figure 5.2). This figure indicates the juice compounds that were statistically significantly affected by the treatments in the respective juices during the processing steps. This overview of compounds that responded to UVB attenuation under certain conditions and juice processing stages also guided the analysis of the individual compound groups.

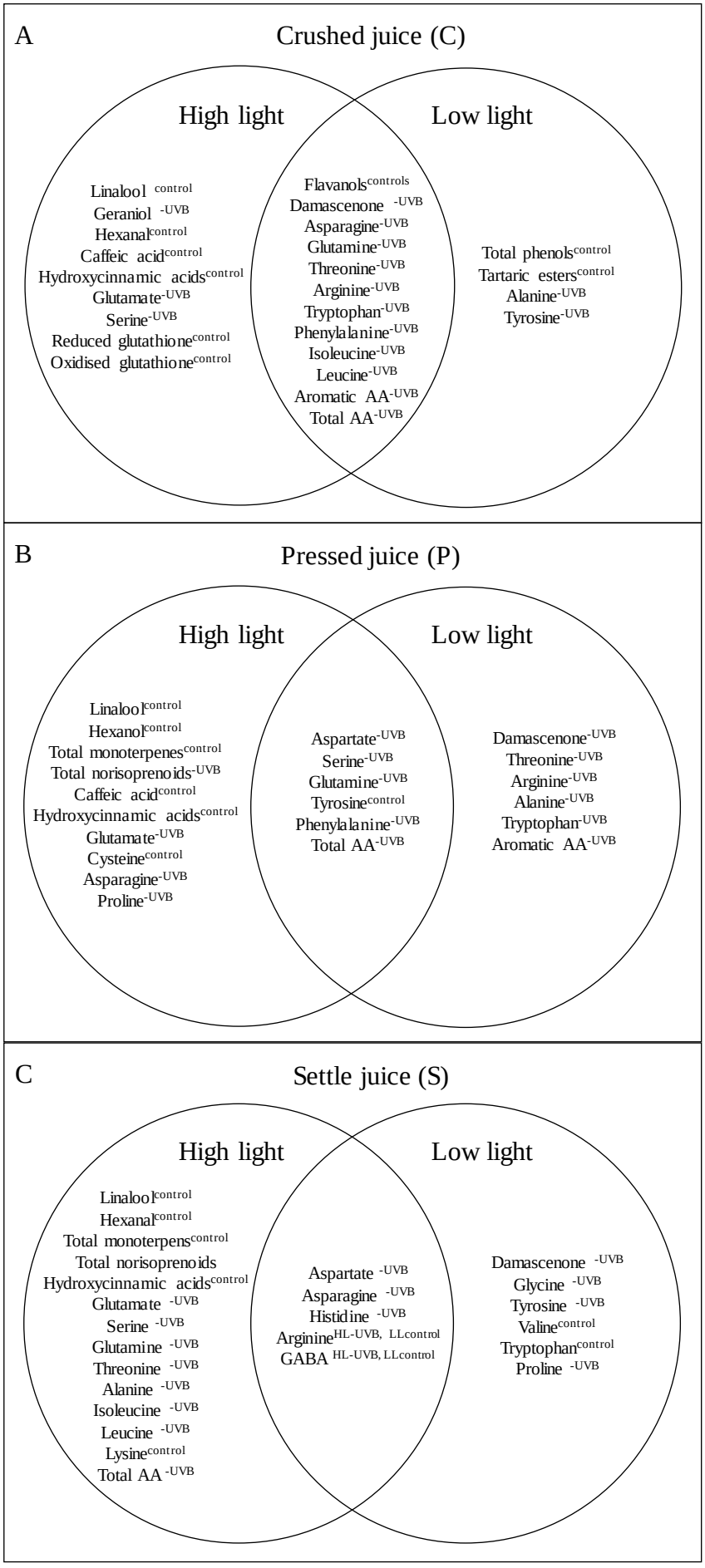


Figure 5.2. A Venn diagram showing the compounds which responded to UVB attenuation in HL and LL microclimates for the crushed juice in panel A, the pressed juice in panel B, and the settled juice in panel C. Compounds were selected based on VIP scores for OPLS-DA models (>0.95) and on significance tested with factorial ANOVA and Fisher LSD Post Hoc tests (adjusted p-value, q-value ≤0.05). The superscripted "control" and "-UVB" indicate in which treatment the specific compounds were statistically increased. In the intersections, "control" indicates a significant increase in the HL and LL controls, similarly "-UVB" indicates a significant increase in the HL-UVB and LL-UVB treatments. In cases where treatment responses are different for the HL and LL microclimate, the full

5.3.2.1 Juice major volatiles

Some of the juice major volatiles presented interesting profiles and the most significantly affected groups/compounds are shown in Figure 5.3. Interestingly, the HL environments had significantly increased levels of total monoterpenes in all stages of juice processing, with linalool contributing prominently to these levels. The UVB attenuation however reduced the levels of total monoterpenes in the HL environment. Hexanal was another example where UVB attenuation in the HL environment caused a decreased in compound levels, specifically in the C-stage samples. Moreover, the hexanal profile of the HLcontrol samples in the different juice processing steps was unique and only showed a slight decrease from the C to S stages, whereas in all other cases the hexanal levels dropped significantly from the first processing step. The total norisoprenoid pools, as well as β-Damascenone increased in all samples through the juice processing steps, reaching the highest levels in the settled juices with the HL-UVB attenuation samples reaching marginally higher levels.

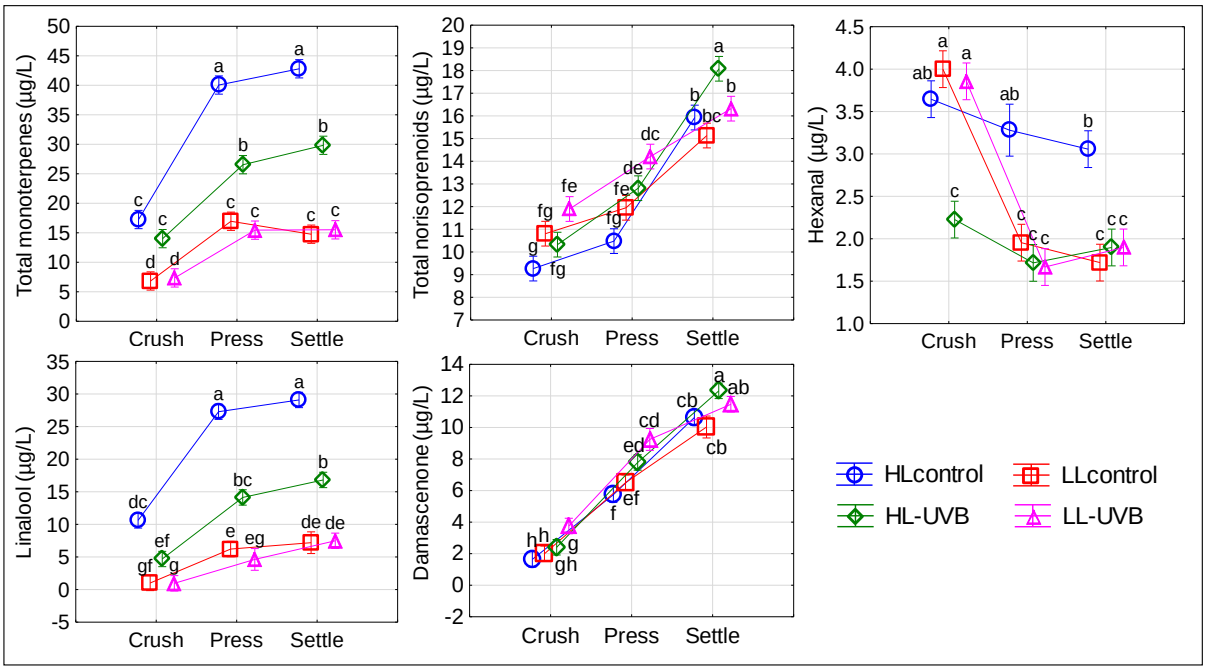


Figure 5.3. ANOVA plots of selected volatile compound groups and the main volatile compounds contributing most to the separation between treatments. Different letters indicate significance according to Fisher LSD post hoc tests.

5.3.2.2 Phenolic analysis of the juice matrices

Interestingly, the total polyphenolic pool quantified in the juices did not respond significantly to UVB attenuation, but did show higher levels in both the HLcontrol and HL-UVB samples when compared to the samples from the LL microclimates. This was mostly due to caftaric acid (Figure 5.4). Similar results were seen regarding the tartaric esters of hydroxycinnamic acids, although, the hydroxycinnamic acids themselves were affected by UVB in the HL microclimate, which showed a significant reduction

with UV attenuation for all juice samples, similar to what was noted in the hydroxycinnamic acid pool. Total flavanols did however show increased levels with UVB attenuation in the HL and LL microclimate for the C-stage samples.

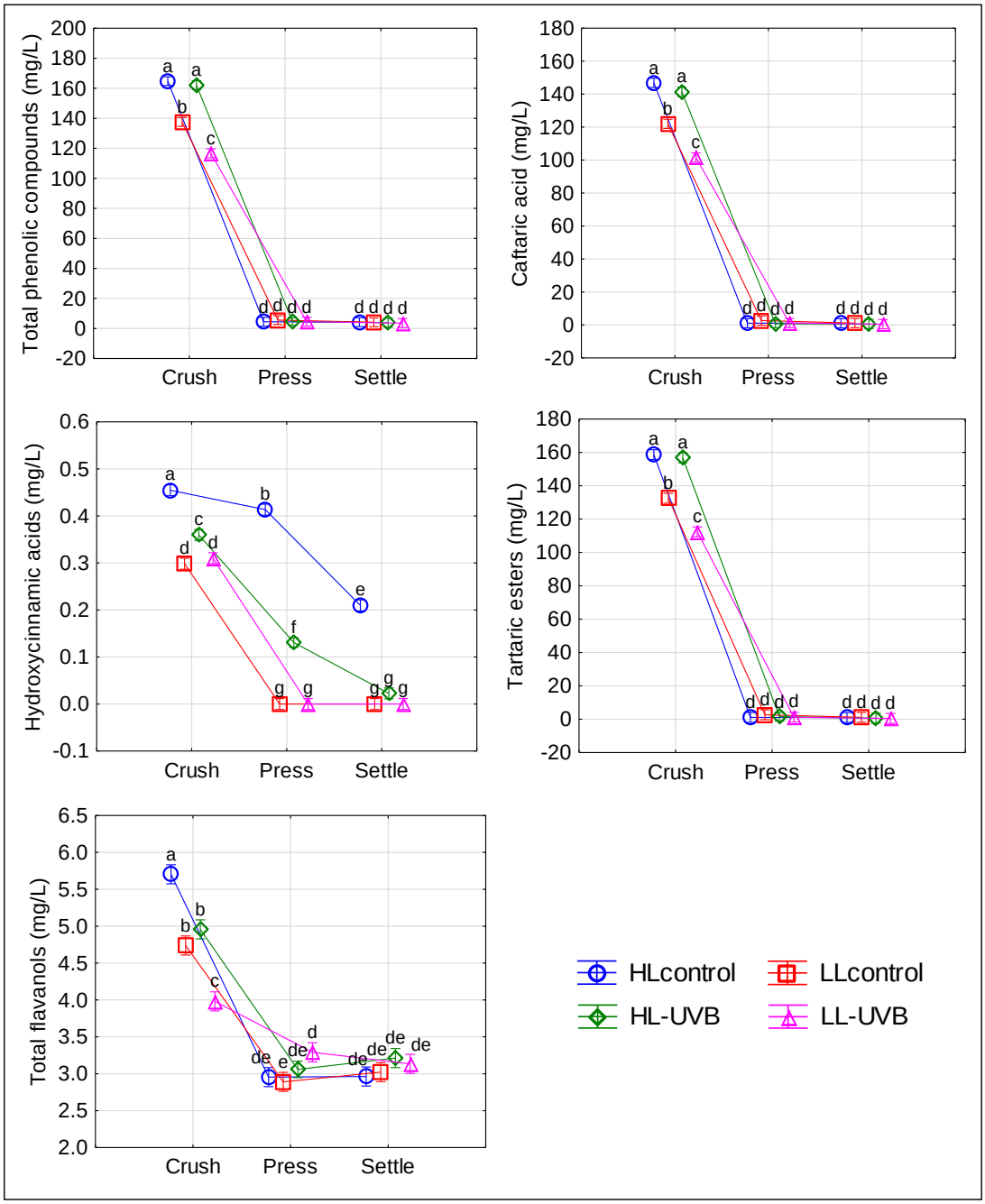


Figure 5.4. ANOVA plots of selected phenolic groups for the four microclimates over the three juice processing steps. Different letters indicate significance according to Fisher LSD post hoc tests.

5.3.2.3 Juice glutathione

An increased abundance of the reduced glutathione in the HLcontrol samples were confirmed, while the LL-UVB displayed the lowest levels. The opposite trends were seen with regards to the levels of oxidised glutathione in the juice samples (Figure 5.5A). Interestingly, these results also corresponded to the visual colour of the juice samples, with the crushed juice showing the least signs of oxidation after defrosting and the HLcontrol juice appeared to display the least visible signs of oxidations throughout the three juice processing steps (Figure 5.5B).

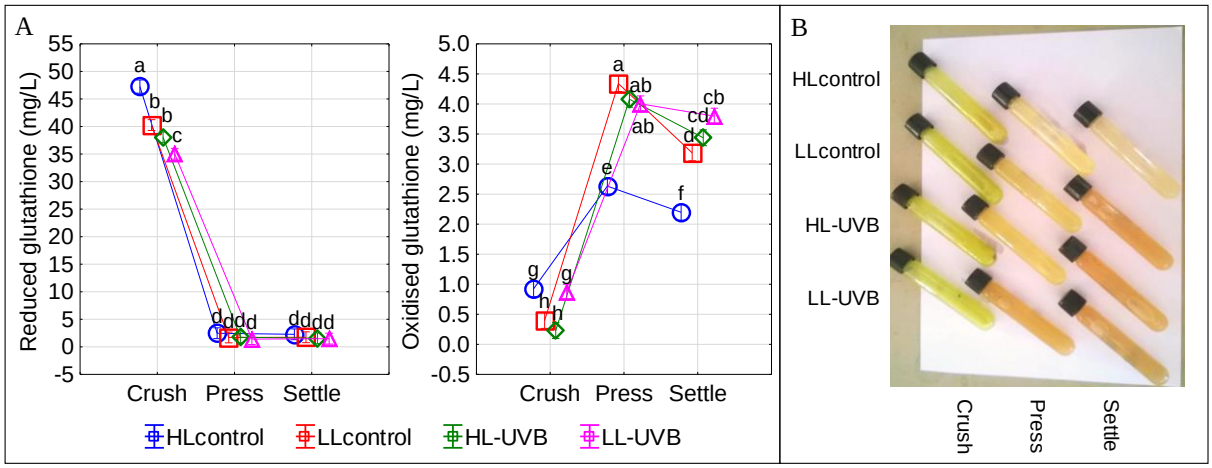


Figure 5.5. ANOVA plots of reduced and oxidised glutathione. Different letters indicate significance according to Fisher LSD post hoc tests (A). Following defrosting, the juices showed different colours which can be related to oxidation (B)

5.3.2.4 Amino acids

The measured amino acids are presented in Supplementary Table 5.1. The data shows that amino acid levels in the juices differed with UVB attenuation under both HL and LL microclimates. Furthermore, changes were seen over the different juice processing stages for each of the four unique juices. This data and the statistically significant differences are summarised and presented in Figure 5.6. Amino acid data was used to create a heatmap to aid in the visualisation of treatment effects for amino acids per juice matrix, which are presented in order of their known preferred utilisation by *Saccharomyces cerevisiae*.

Most of the amino acids showed a significantly lowered level in the HLcontrol samples, most notably in glutamic acid, glutamine and serine (Figure 5.6). Phenylalanine, leucine and isoleucine were further significantly affected by UVB attenuation, particularly in the HL-UVB microclimate, which displayed increased levels across the juice sampling points. In the LL microclimate, UVB attenuation led to increased phenylalanine in the pressed juice and higher leucine levels in the crushed and settled juice. These amino acid are all known to be consumed early by *S. cerevisiae* (Crépin et al., 2012).

The amino acids which are known to be consumed later by *S. cerevisiae* also responded differentially to the variation in light exposure and UVB attenuation over the three sampling points. Arginine and alanine, were observed to be significantly decreased in the HL control samples, specifically in the crushed and settled juice samples.

The non-preferred amino acids proline and GABA increased in levels during juice processing. Regarding proline, the HL-UVB samples showed a consistent increased content across all sampling points, whereas UVB attenuation only led to higher levels in the settled juice of the LL environment. GABA also showed a complex profile with higher levels in the HL juice samples versus the LL juices, however, within these microclimates in the settled juice, UVB attenuation was seen to lead to increased levels of GABA in the HL microclimate, whereas in the LL microclimate the control instead increased in GABA content (Figure 5.6).

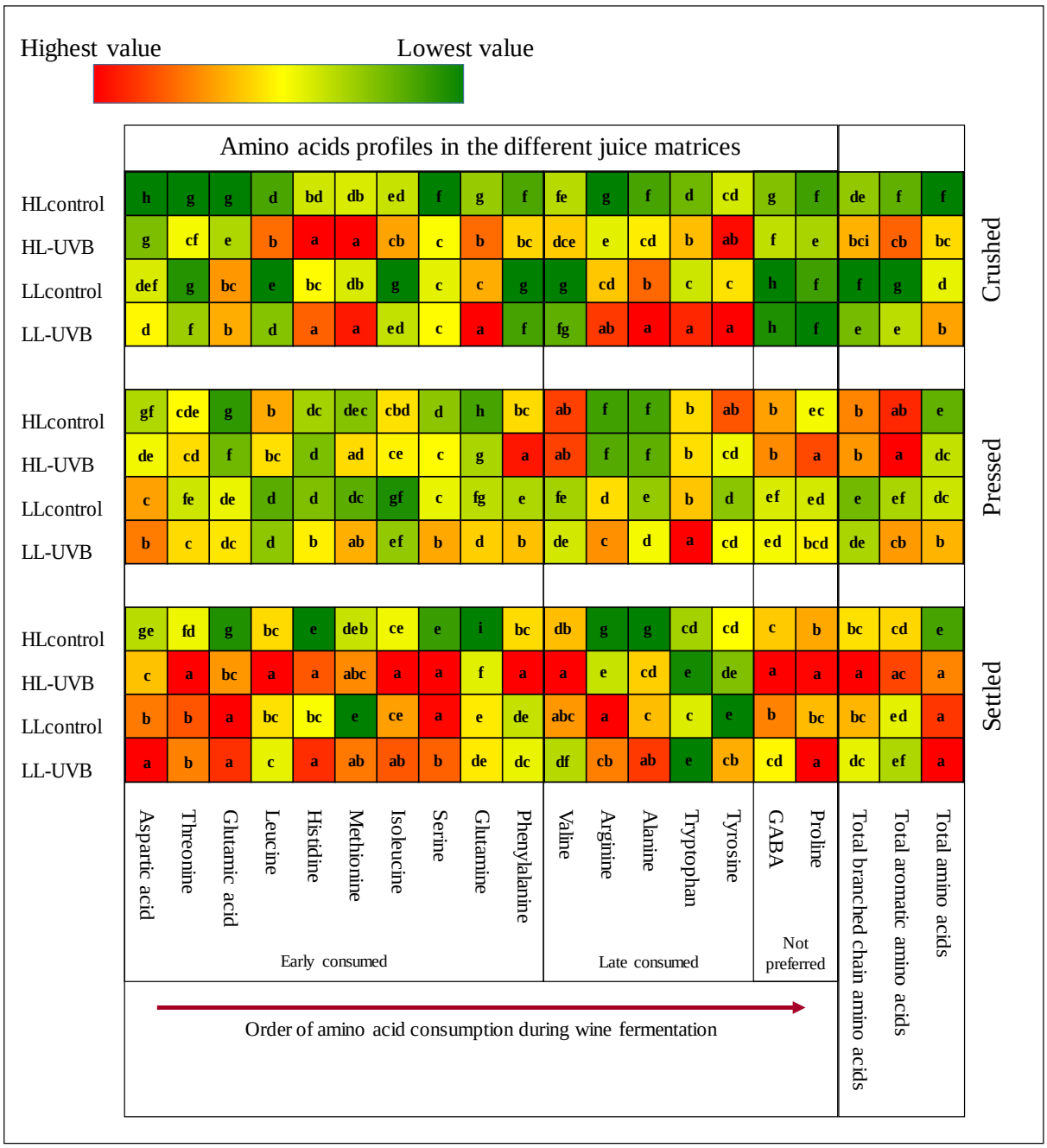


Figure 5.6. A heatmap representing the metabolic responses of the amino acids seen in the three juices. Each block represents the average of the measured amino acid content (mg/L) for each treatment in the different juices. Values are scaled individually for each amino acid over the three juices from highest to lowest by colour. Red indicates the highest amino acid value, while green indicates the lowest amino acid value for that specific compound (column).The different letters indicate significant differences as presented in the factorial ANOVA plots and Fisher LSD post hoc tests. These are indicated per amino acid over the different juice matrices to indicate significance between treatments over the three juice processing stages.

5.3.3 Hierarchical clustering analysis

The clustering analysis was used to identify metabolites/compounds that share similar patterns during juice processing and is presented in Figures 5.7 and 5.8. The majority of the metabolites (27 metabolites) were affected by juice processing (Figure 5.7) and the biological repeats were also shown to cluster according to their sampling groups. Within the C and P juice clusters, samples grouped first according to light exposure, whereas UVB attenuation formed smaller clusters within these HL and LL groups. The S juice samples, however clustered more according to UVB attenuation, with the HL-UVB and LL-UVB samples grouping together. Interestingly, the HL control S juice clustered with the P juice samples (as annotated by the red block in Figure 5.7) showing strong similarities to the HL S juice sample. Juice processing stage was therefore confirmed to be the dominant factor driving this analysis, obscuring the effects of light and UVB.

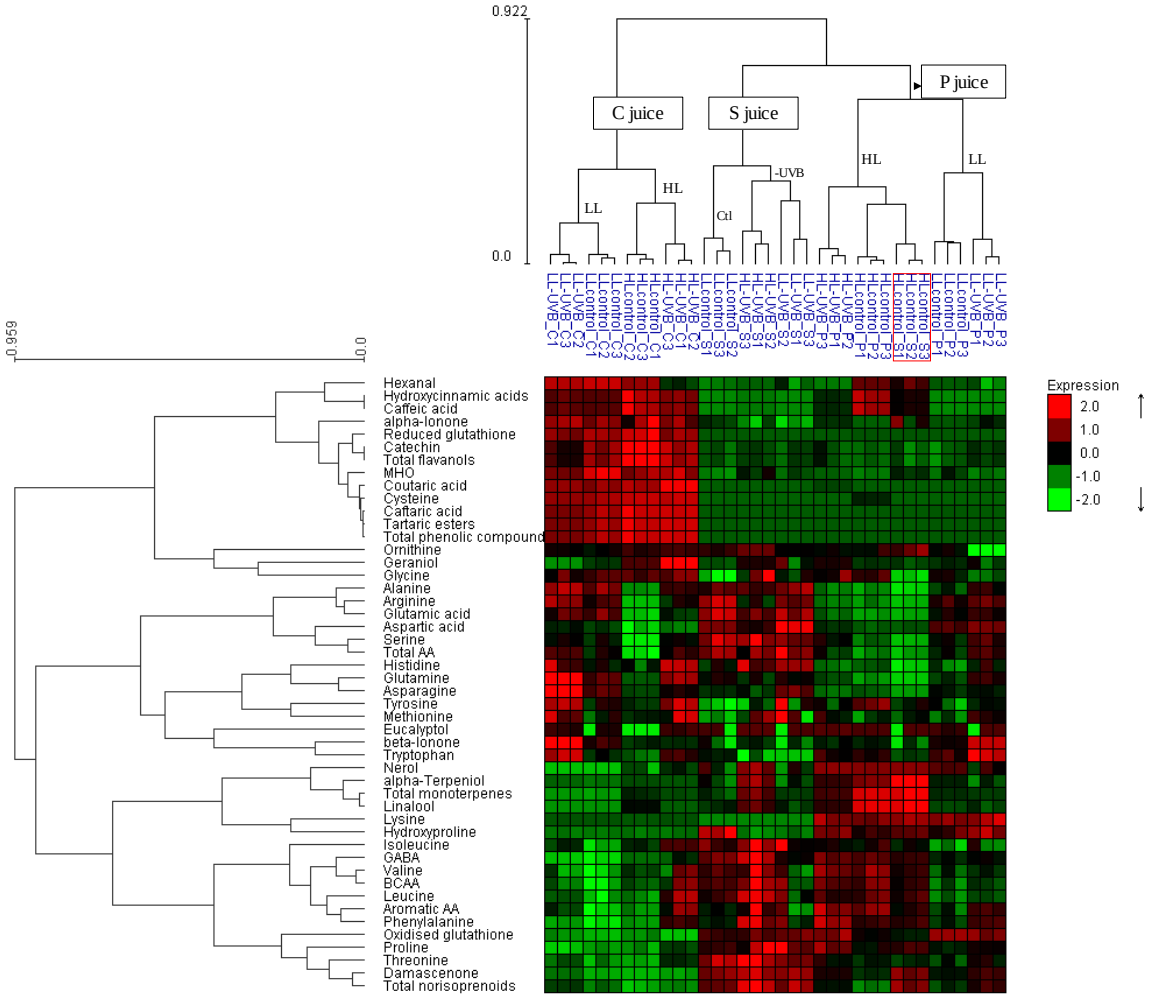


Figure 5.7. Hierarchical cluster analysis of all variables from the four microclimates measured in all juice stages. Data was clustered by metabolite and sample. Significant cluster groups are indicated on the figure (Ctl = Control). The red border surrounds a group of technical repeats which do not fit into the major P juice cluster.

By grouping compounds with similar profiles together, six clusters were identified (Figure 5.8). Cluster 1included 8 compounds which increased with juice processing stage and seemed to be responsive to UVB in the HL microclimate including the amino acids aspartic acid and threonine as well as oxidised glutathione. Cluster 2 contained 19 metabolites that decreased with juice processing stages; including the polyphenolic compounds, some volatile compounds including geraniol, hexanal and MHO as well as reduced glutathione. The majority of compounds were found in these two clusters, again reiterating the significant effect of juice processing stage on the measured compounds. However, some UVB and light responses were seen. In cluster 3 a strong UVB attenuation influence in the HL samples was seen and included mostly amino acids such as glutamic acid, glutamine, alanine and arginine. Cluster 5 (7 compounds) also exhibited clear UVB attenuation differences, most notably in the HL samples. Metabolites in this cluster included the branched chain amino acids (valine, leucine and isoleucine), phenylalanine and GABA. Finally, cluster 6 contained 5 compounds which responded to UVB in the HL microclimate specifically and included linalool, α-terpineol and the monoterpene pool.

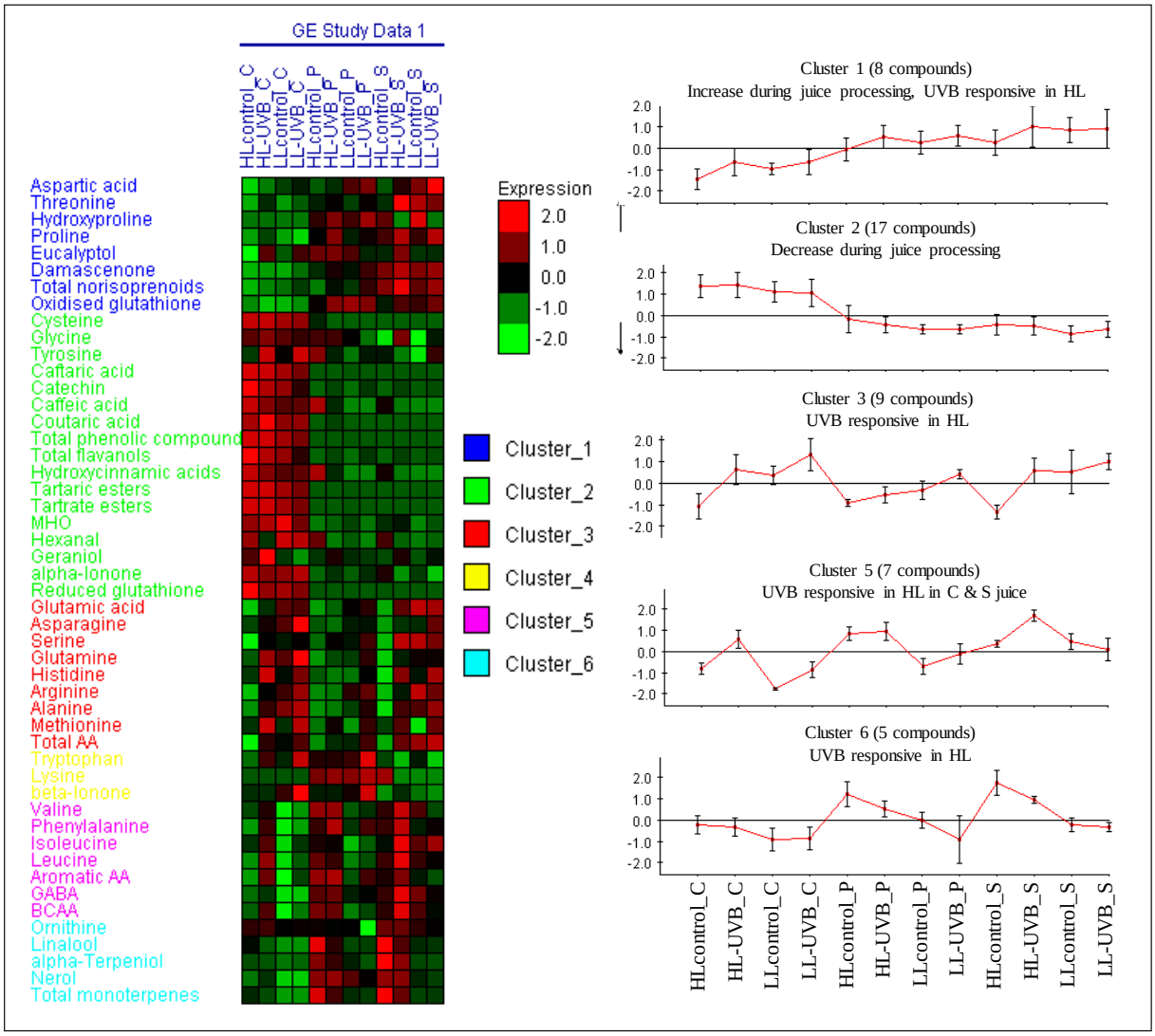


Figure 5.8. The cluster expression matrix and important cluster mean patterns for the measured compounds from the four microclimates measured in the three juice matrices. Compounds with similar correlative patterns were grouped together. These were clustered on the metabolite level.

Cluster analysis was also conducted separately on each juice processing stage. The results are shown in Supplementary Figures 1 and 2 of Chapter 5 and highlighted light exposure as the main factor clustering the data. Interestingly and conversely to the other juice matrices, hierarchical clustering revealed UVB attenuation as the main driver in the metabolite profiles in the settled juice samples as shown in Figure 5.9.

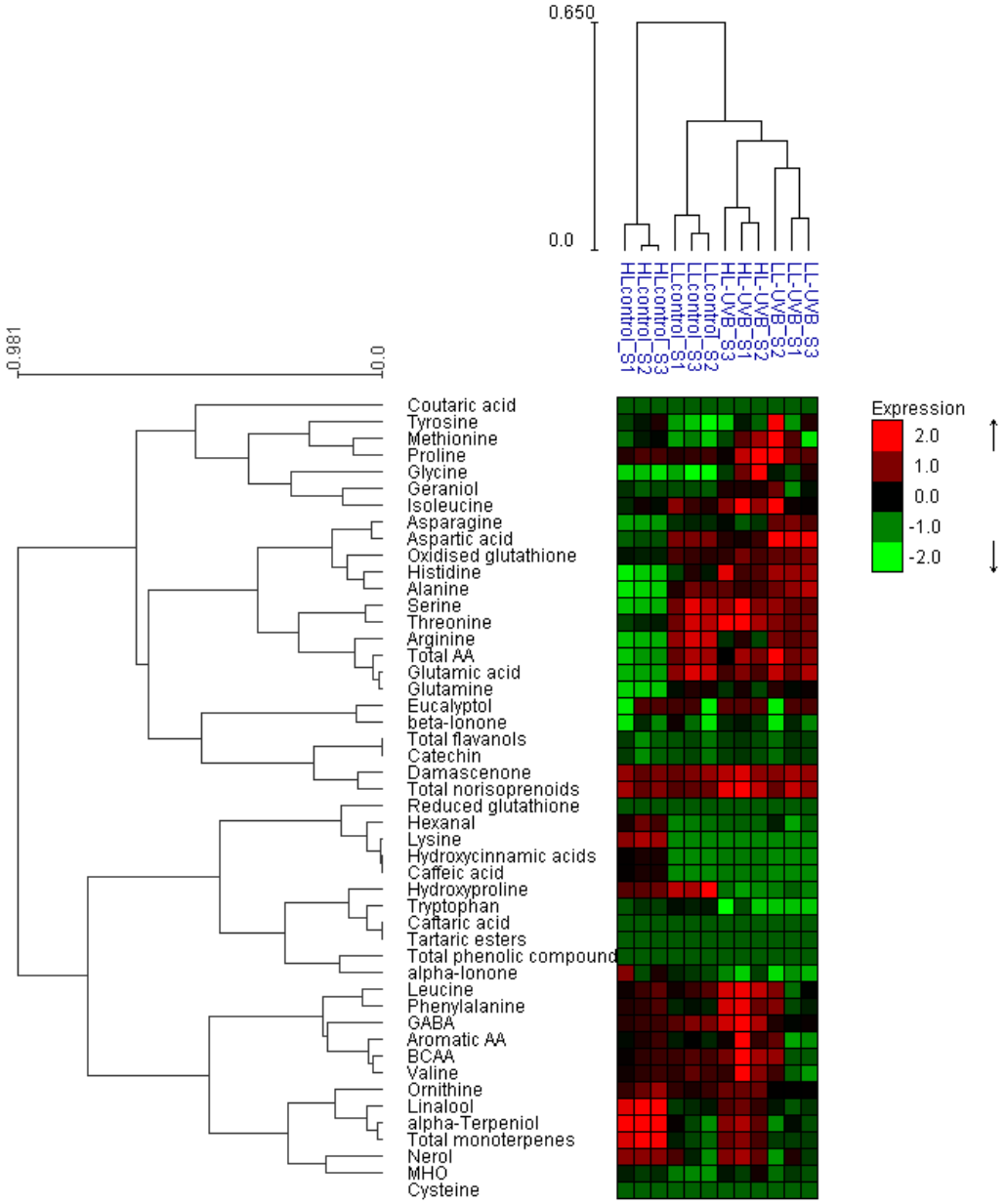


Figure 5.9. Hierarchical cluster analysis of all variables from the settled juice measured in the four microclimates.

Cluster patterns and the associated cluster expression matrix highlighted those compounds, which responded to UVB attenuation (Figure 5.10). Cluster 1 contained 11 compounds, which were less expressed in the HLcontrol versus HL-UVB samples, including a number of amino acids such as

arginine, alanine, glutamine and glutamic acid, the total amino acid pool as well as oxidised glutathione. Furthermore, similar expression was seen between the HL-UVB and the LL samples. Conversely cluster 2 included compounds which were higher in the HLcontrol compared to the HL-UVB samples and included a number of polyphenolic compounds, reduced glutathione as well as certain volatiles such as linalool, α-terpineol and the monoterpene pool. Cluster 3 compounds, including the phenylalanine, valine, leucine as well as the aromatic and branched chain amino acid pool showed a UVB response in the HL microclimate specifically (higher in HL-UVB), with the LL samples being expressed similarly to the HLcontrol samples. Finally cluster 5 included 8 compounds which were higher in the reduced UVB samples for both the HL and LL microclimates. These compounds included tryptophan and caftaric acid.

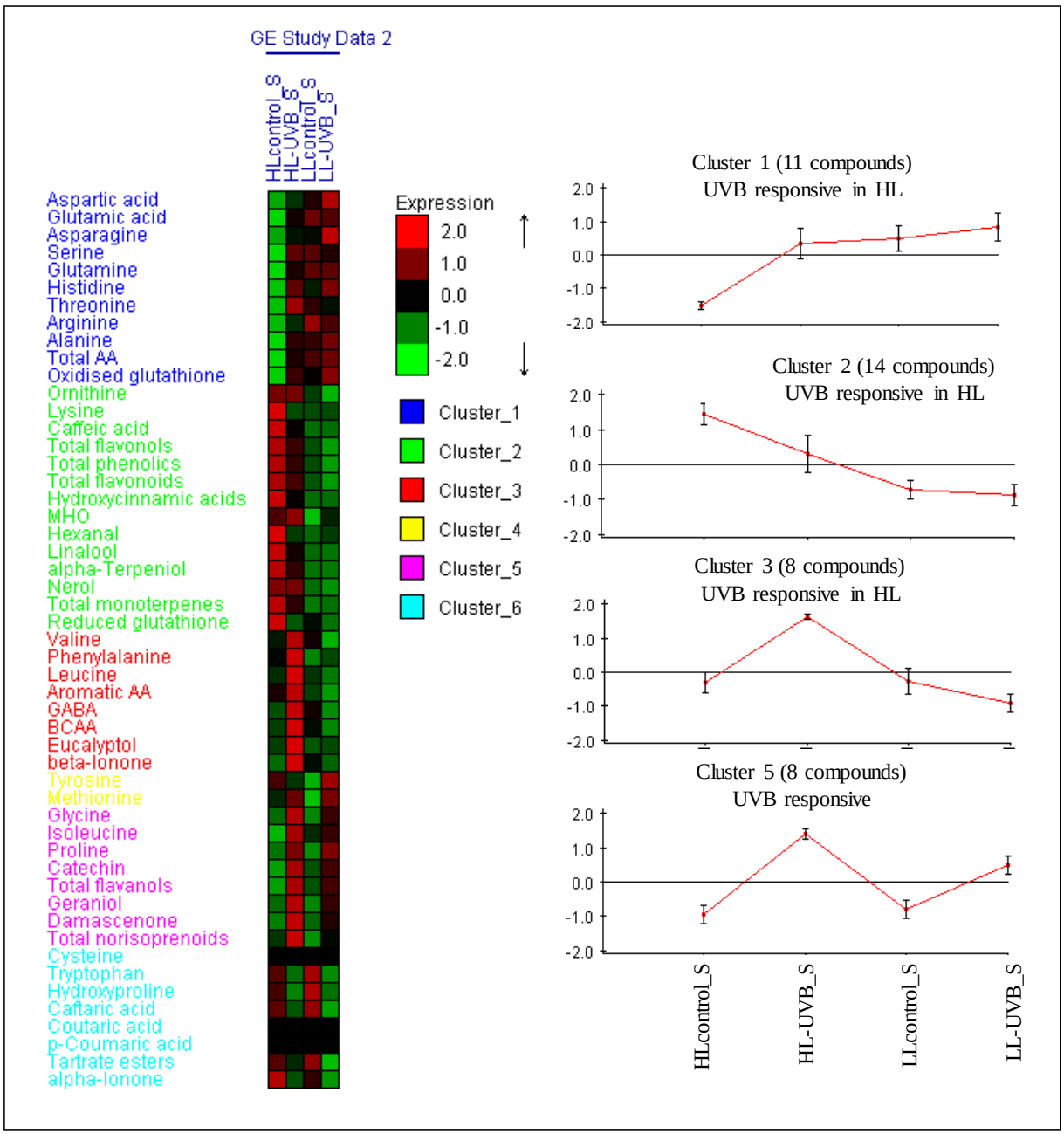


Figure 5.10. The cluster expression matrix and important cluster mean patterns for the measured compounds from the four microclimates measured in the settled juice matrix. Compounds with similar correlative patterns were grouped together. These were clustered on the metabolite level.

5.4 Discussion

A number of studies have looked at the effects of abiotic factors on the grape berry, particularly at the ripe stage of development and how these potentially affect the wine, also in Sauvignon Blanc (Antalick et al., 2015; González-Barreiro et al., 2015; Martin et al., 2016; Robinson et al., 2014; Suklje et al., 2014). A better understanding of these berry responses has allowed for the development and application of different viticultural management practices to manipulate the grape berry characteristics and thereby alter the resulting wines. Despite the efforts made to better understand the links between the vineyard and the wine, very few trials have been conducted on the juice, particularly in terms of light exposure

and UVB radiation. The results of this study revealed interesting juice responses, not only to the attenuation of UVB with differential light exposure, but also during processing, prior to fermentation. These impacts are potentially significant in modulating wine characteristics, highlighting the importance of juice assessment.

Considering the basic juice analysis for sugars, acids and pH (Table 5.1), the juices were quite similar over the seasons between the treatments, showing an insignificant effect of UVB attenuation on juice sugars and acids in both the HL and LL microclimate. Chapter 3 showed a similar result in the ripe grape berries, indicating that UVB did not affect primary metabolism during ripening in this study. In season 3, during which a more intense investigation of the juice was carried out, similar sugar levels were observed across the different juice processing steps, however a slight drop in acids was observed in the pressed and settled juices. It has been noted that wine produced on the stems and seeds leads to lowered titratable acidity, presumably due to the leaching of potassium from said stems and seeds which precipitates a fraction of tartaric acid in the form of potassium hydrogen tartrate (Katsumi Hashizume et al., 1998; Pascual et al., 2016). It is possible that during the 24 hour skin contact, acids were in a similar manner precipitated out due to the presence of the grape pomace, resulting in a lowered TA in the crushed and settled juice across all samples.

Despite the evident similarities over the seasons in terms of basic juice analyses, the other measured compounds including amino acids, phenolics, volatiles and oxidation status of the juices showed different responses and therefore presented interesting perspectives as well as confirming the influence of the four microclimates on the juice characteristics.

5.4.1 Oxidation potential of the four juices

The results revealed a significant influence of the microclimate on the glutathione and polyphenolic composition of grape juice. These compounds are known to be involved in juice oxidation status, suggesting that light quantity and quality can influence these aspects, which are influential in the process of fermentation and in determining final wine characteristics. Most notably, exposed berries with ambient UVB exhibited the highest glutathione levels at the crushed juice stage, however the levels of reduced glutathione dropped significantly following pressing in all juice samples. Similar results were reported in Suklje et al (2014) who found higher levels of glutathione in Sauvignon Blanc grape must from berries exposed to ambient UVB levels. Glutathione is a non-enzymatic antioxidant, which contributes to browning prevention in grape juice (Wu, 2014), however it is found in grape berries and wine as well. The reduced form is the most common form in juice and this compound helps to protect the juice from oxidation and thereby lessens browning. Oxidised glutathione is not present in high levels and can be reduced back to glutathione (Kritzinger et al., 2013). Interestingly, although the reduced form of glutathione appeared to drop to insignificant levels, the oxidised form was seen to be much lower in the exposed juice (HLcontrol) compared to the other samples, implying a reduced degree of oxidation

in the HLcontrol juice. This correlated well with the visible degree of browning seen in the different juice samples used for analysis (Fig 5.5). It is important to note that SO_2 was added to the juices during crushing, thereby providing a degree of protection from oxidation. The elevated glutathione levels in the HLcontrol juice most likely provided further protection once the SO_2 levels became insufficient, thereby reducing juice browning in these samples.

Further implicated in the juice oxidation status is the presence of polyphenolic compounds. Many trials have been conducted specifically on the effects of light quantity and quality on grape phenolic compounds due to their importance as secondary metabolites in the berries and as quality impact compounds in the wine (Condurso et al., 2016; Downey et al., 2006; Price et al., 1995; Ristic et al., 2007; Song et al., 2015). Here, in general, the polyphenolics were increased in the juices under HL conditions. This was similarly seen in the study by Martin et al. (2016). These compounds have been related to the antioxidant potential of grape juices and will influence juice oxidative status. The variations noted in these compounds may therefore also have contributed to the colour preservation of HLcontrol juices, similarly to glutathione.

Interestingly, a significant decrease was seen in polyphenols in all the juice samples following pressing and the consequent removal of the pomace, noting specifically caftaric acid. The caftaric acid levels decreased significantly between the crush juice and press juice samples for all microclimates. This is potentially due to oxidation as caftaric acid is the main phenolic compound which is oxidised in juice to form the GRP with GSH (Coetzee et al., 2011). This is supported by the noted drop in glutathione as well. To confirm this, it would have been prudent to measure the GRP as a way to confirm this hypothesis. If oxidation did occur to this degree, it could also explain the drop in levels of the other phenolic compounds. This significant drop in phenolic compounds may however also be related to the absence of the skin and seed tissue as these compounds are predominantly found in this fraction (Pinelo et al., 2006; Rodriguez Montealegre et al., 2006). It is possible that the phenolics adsorbed to cell walls of the berry tissue, which are generally removed during pressing. This reaction would involve the formation of polyphenol – cell-wall complexes which can occur through the non-covalent adsorption of polyphenols to the cell wall matrix (recently reviewed in Renard et al., 2017). The hydroxycinnamic acids however were somewhat maintained throughout juice processing in the exposed samples with higher levels in the control juice. In an earlier study by Renard et al. (2001), using apple to investigate the interactions between polyphenols and cell walls, it was shown that hydroxycinnamic acids did not bind to the cell wall material. A similar response may have occurred here in the crushed juice matrix, thereby limiting the removal of these compounds from the juice following pressing and the associated removal of skins and seeds. Furthermore, hydroxycinnamic acids are localised to both the skin as well as the flesh of grape berries (Licker et al., 1998), perhaps making them more abundant in grape juice following removal of the skin and seed tissues. Both these hypotheses however would be related to the amounts inherently found in the grape at harvest, therefore suggesting a higher content in the exposed

berries. Hydroxycinnamic acids have been shown to accumulate more under conditions of elevated light exposure (Koyama et al., 2012b; Sun et al., 2017) explaining the higher levels found in the more exposed berries. Furthermore, hydroxycinnamic acids may serve as UV screens in plants as has been discussed in particular publications (Fabón et al., 2010; Kolb et al., 2001; Landry et al., 1995b). Due to their ability to effectively absorb radiation in the UVB spectral range, hydroxycinnamic acids are effective at screening UVB (Sheahan, 1996).We propose that attenuated UVB may have resulted in a reduced accumulation of hydroxycinnamic acids in the ripe berries, which may have translated to the lowered levels seen in the corresponding juices.

5.4.2 The amino acid profiles at the different juice processing stages provides a glimpse into the dynamic nature of the juices as well as the potential impact of bio-transformations on these compounds following harvest and prior to fermentation

Firstly, significantly different amino acid profiles were noted in the samples taken at berry crushing (C samples) between the different treatments (Figure 5.2 and 5.6), confirming that the four microclimates created four different juices in terms of amino acid composition. Strong treatment effects were also seen in the amino acid profiles of the ripe berries (Chapter 4) and the extraction and release of the compounds into the juice matrices, combined with possible biological or chemical processes will have contributed to the distinctive composition of each juice. Analysis of the samples taken at each of the juice processing stages showed that the amino acids displayed dynamic trends, both increasing and decreasing at different stages and ultimately leading to a compositionally different settled juice when compared to the crushed juice. This suggests the activity of chemical and/or biological processes capable of incorporating or releasing amino acids in the juice during processing prior to fermentation. Although no enzyme assays or protein content evaluation occurred in this study, our data motivates for such analyses to better understand the underlying processes that contribute to this complex matrix.

Some biological activities are likely to occur in the juice matrices, for example, processing steps may release natural grape-derived proteases which can break down proteins to yield amino acids (Van Rensburg and Pretorius, 2000). Plant cell vacuoles are known to house numerous hydrolytic enzymes such as proteases (Boller and Kende, 1979; Eisenach et al., 2015; Vitale and Hinz, 2005; Zamyatnin, 2015). The disruptive crushing and pressing events during juice processing break open grape cell vacuoles, thereby releasing their associated enzymes. Other enzymatic activities may also lead to the aggregation of amino acids in protein formation, thereby reducing their presence in the juice. Furthermore, climatic conditions during berry development may have influenced protein formation and abundance, consequently also affecting juice proteins, which in turn may be related to amino acid levels. The natural microbiome of the grapes could also influence protein abundance and amino acid composition through their natural enzymatic capabilities (Van Rensburg and Pretorius, 2000).

Considering the impacts of the four microclimates, during processing, significant influences of light and UVB were seen on the amino acid levels and profiles. Most notable, it was observed that the HLcontrol juice samples consistently contained lower levels of total amino acids, meaning that amino acids were either incorporated into proteins or lower levels were available/extracted from the grape berries. du Plessis et al. (2017) showed that berries exposed to increased light in this vineyard probably utilised certain amino acids to maintain the energy metabolism, while transcriptomic analysis indicated increased turnover of proteins as one of the acclimation responses.

Interestingly, under both HL and LL conditions, UVB attenuation resulted in an increase in the aromatic and branched chain amino acids (Figure 5.6 and 5.8), most notably isoleucine, leucine and phenylalanine (Figure 5.2), in the juice at crushing. When the settled juice was analysed however, the only significant effect was a higher incidence of branched chain amino acids in the HL-UVB samples (Figure 5.10). Since these amino acids form the most important odour-related products, including certain higher alcohols and/or volatile fatty acids, their presence in the settled juice could influence the formation of these important organoleptic compounds, thereby influencing wine characteristics (refer to Chapter 6 for wine and sensory analyses of the wines made from these treatments). Given the importance of the amino acids for yeast metabolism during the wine fermentations, the amino acid profiles were also presented in terms of the correlation of the abundance of the individual amino acids and the known uptake preference by *S. cerevisiae* (Figure 5.6). It suggests that both light exposure and UVB attenuation could possibly influence the fermentations, an aspect that will be discussed in Chapter 6.

5.4.3 The aromatic potential of the four juices were different and changed during juice processing

The aromatic potential of the juices were compared with a particular focus on grape-derived aroma compounds, barring the thiol precursors, for which an analytical method was not available in our environment at the time of the experiments.

As observed for the amino acids, the grape-derived volatile compounds measured showed interesting and dynamic patterns in the different juice matrices in relation to the treatments. The HL environments showed elevated levels of monoterpenes and norisoprenoids with UVB attenuation differentially affecting their levels. Significantly higher levels of certain monoterpenes, including linalool were measured in the more exposed grapes at harvest, with attenuated UVB leading to reduced levels; these results were discussed in Chapter 3. These differential patterns carried through to the initial stage of the juice processing, indicating that the grapes that had higher levels of volatiles also yielded juices enriched in these compounds. For example, at the crushing stage linalool levels were significantly higher in the HLcontrol samples, followed by the HL-UVB samples, whereas shaded berry juice showed the lowest linalool content. This also confirmed results presented by Sasaki et al. (2016). Overall, the volatile profiling highlighted the significant influence of UVB on juice volatile composition, and confirming that light exposure further differentiates these UVB responses.

The monoterpene levels increased from the crushed stage to the settled juices (Figure 5.3). Monoterpenes are typically localised to the skin (Cabrita et al., 2006; Gunata et al., 1985; Slegers et al., 2015; Ugliano et al., 2006) and have been shown to accumulate in response to external stresses such as UVB (Bureau et al., 2000; Carbonell-Bejerano et al., 2014; Song et al., 2015; Zhang et al., 2014) (also discussed in Chapter 3). Skin contact has been shown to increase the levels of monoterpenes in juice (Lukić et al., 2017; Marais, 1998) and at least part of the increased levels (and differential levels between treatments) could have been due to the leaching of the compounds during the juice processing stages. Furthermore, as the juice processing stages progressed, enzymatic action would have released the glycosidically-bound monoterpenes, leading to the increasing levels in the settled juices. The comparable accumulative trend noted in the norisoprenoids may be similarly explained as they are also to a certain extent bound to the skin tissue fraction (Chen et al., 2017). Both the monoterpenes and norisoprenoids contribute to wine aromatic characteristics and their presence in the juice could therefore significantly impact the final product. These compounds typically remain unchanged by yeast metabolism and persist in the finished wine (Black et al., 2015; Hock et al., 1984; Mateo and Jiménez, 2000).

5.5 Conclusions

The approach taken here to profile the juices throughout the three juice processing steps provided interesting insights and, in our opinion, novel insights into the dynamic nature of this arguably under-studied wine matrix. Juice studies in Sauvignon blanc specifically are scarce with only a few recent studies becoming available, most notably in relation to thiol precursors and development in wine (Araujo et al., 2017; Martin et al., 2016; Thibon et al., 2016). This is the first study to our knowledge which has characterised the juice matrices during processing in relation to variations in UVB radiation under HL and LL microclimates. This study enabled the identification of certain compounds which were transferred unchanged from the grape to juice matrices, whereas other compounds such as the amino acids displayed a more labile compositional profile in the different processing steps. These compounds are therefore potentially involved in complex reactions and bio-transformations that underlie the dynamic patterns observed as the juice processing proceeded. Despite these complex transitions, clear exposure-related and/or UVB attenuation-responsive compounds could be identified. Furthermore, the characterisation of the juices as well as the observed profiles could possibly provide links that relate back to the characterised berries as well as help identify key metabolites which relate to the final wines.

5.6 References

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Supplementary data to Chapter 5

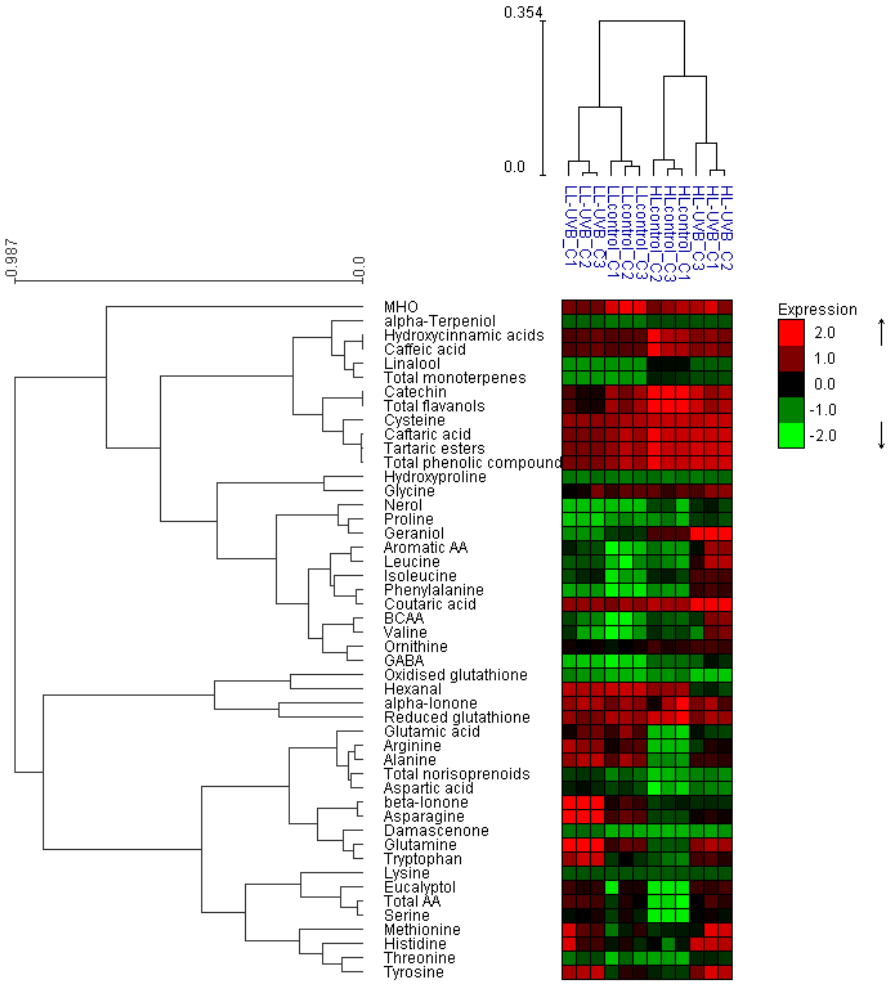
Supplementary table 5.1. A table listing the measured contents of all the compounds ± SD for the three juices. The corresponding p-values between the HL control/ HL-UVB and LL control)/LL-UVB contrasts are calculated and listed for each compound

	Crush juice					
Compound	**HLcontrol**	**HL-UVB**	**p-value**	**LLcontrol**	**LL-UVB**	**p-value**
Amino acids (mg/L) (2014/2015)						
Aspartic acid	19.1±1.2	23.6±2.1	0.03	29.7±2.2	33.4±4.2	0.09
Glutamic acid	131.9±3.5	153.6±5.8	0.00	171.6±6.5	169.6±8.2	0.66
Cysteine	80.3±2.5	79.1±4	0.58	71.5±4.7	66.4±2.4	0.04
Asparagine	7.9±0.1	9.5±0.2	0.00	10.8±0.4	15.8±0.3	0.00
Serine	69.5±0.9	84.4±0.8	0.00	83.1±2.5	84.8±1.6	0.18
Glutamine	161.8±3.6	220.2±6.8	0.00	203.7±8.7	250.7±4.9	0.00
Histidine	48.8±4.4	61.4±3.7	0.00	50.9±4.4	57.3±5.2	0.04
Glycine	6±0.3	6.2±0.3	0.38	6±0.2	5.8±0.7	0.50
Threonine	84.7±0.7	93.4±0.7	0.00	86±2.8	90.1±2	0.01
Arginine	480.6±19.7	614.7±39.9	0.00	671.3±42.9	744±33.1	0.01
Alanine	252.5±2.1	332.5±65.4	0.00	316.6±9.4	329.5±6.6	0.02
Tyrosine	23±0.3	28.9±4.5	0.00	23.9±1	26.8±0.5	0.00
CY2	28.9±3.5	25.5±2.7	0.14	23.5±1.9	23.7±1.6	0.86
Valine	62.7±2.1	51.9±30.2	0.43	56.3±4.5	59.7±5	0.25
Methionine	11.6±0.5	30.6±35.2	0.06	11.6±1	12.9±0.8	0.03
Tryptophan	11±0.6	14.3±0.9	0.00	12.5±0.9	16.9±1	0.00
Phenylalanine	22.7±0.5	29.9±0.7	0.00	19.9±0.7	22.4±0.8	0.00
Isoleucine	15.8±0.3	17.6±0.2	0.00	13.7±0.5	15.8±0.5	0.00
Ornithine	28.5±1.4	29.7±1.6	0.25	25.6±1.5	26.3±0.9	0.32
Leucine	21.1±0.9	26.7±1.9	0.00	19.6±1.6	22.3±1.5	0.01
Lysine	3.6±0.4	4.1±0.3	0.10	3.8±0.3	3.9±0.4	0.82
Hydroxyproline	122.4±22.4	124.9±16.4	0.85	120.2±13.8	119.1±18.3	0.91
Proline	290.9±29.8	360.7±32.3	0.01	289.8±21	248.4±14.9	0.00
GABA	157±6	208.8±73.5	0.05	124.5±5.8	132.1±2.9	0.02
Total aromatic amino acids	119.4±2.3	125±27	0.00	112.6±5	125.8±3.4	0.00
Total branched chain amino acids	99.7±2.1	96.1±31.4	0.02	89.6±5	97.8±4.8	0.02
Total amino acids	2010.3±44	2507.3±127	0.00	2325.8±79	2458.6±25	0.00
Volatile compounds (µg/L) (2014/2015)						
Eucalyptol	<LOD	1.2±0.1		1	1.1±0.1	0.62
6-MHO	4.6±0.5	4.9±0.7	0.67	5.5±0.2	4.3±0.1	0.02
Hexanol	3.6±0.1	2.2±0.2	0.01	4±0	3.9±0.1	0.09
Linalool	10.6±0	4.7±0	0.00	1±0.2	1±0	0.74
α-Terpeniol	1.7±0	1.6±0	0.05	1.4±0.1	1.5±0	0.11
Geraniol	4.1±0	4.6±0.1	0.02	3.9±0	3.8±0	0.01
Nerol	1.6	1.9±0.4	0.64	<LOD	<LOD	
Damascenone	1.6±0	2.4±0.4	0.11	2±0.3	3.8±0.2	0.02
α-Ionone	1.2±0.1	1.2±0	0.79	1.2±0	1.2±0	0.40

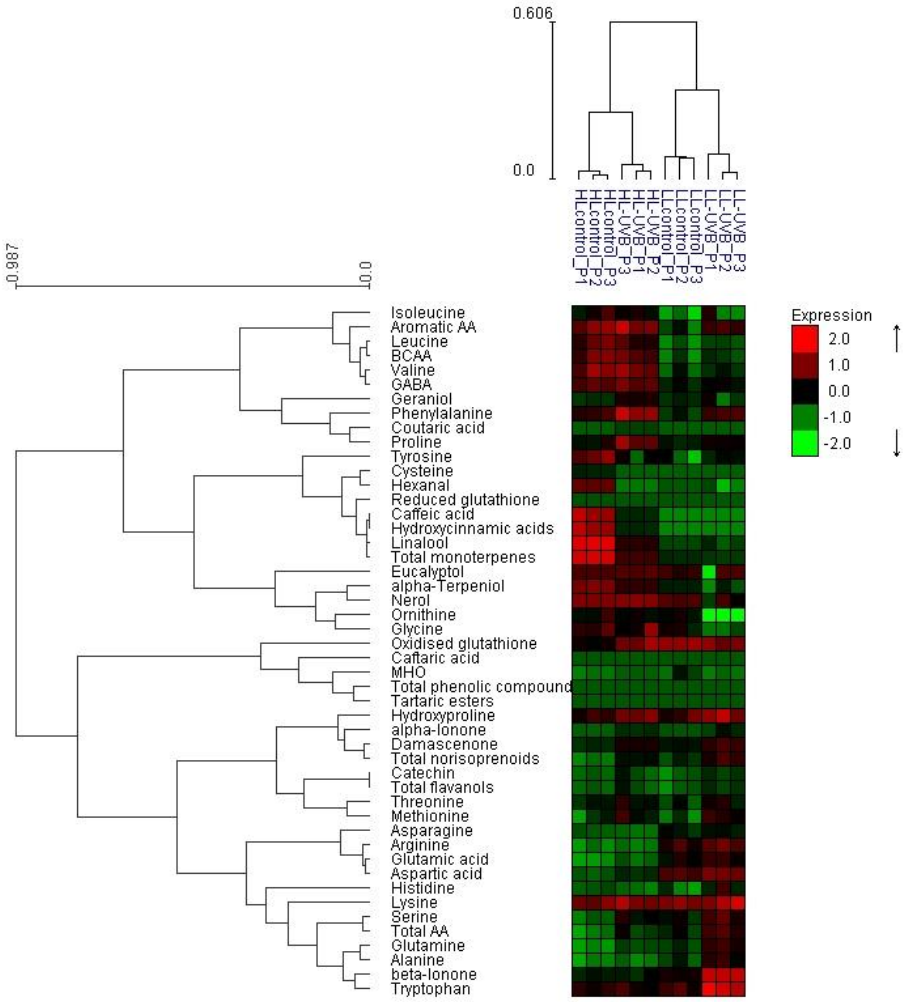
β-Ionone	1.8±0.1	1.8±0	0.97	2.1±0.1	2.7±0.1	0.02
Total monoterpenes	17.3±1.2	14±0.5	0.07	6.8±1	7.3±0.2	0.55
Total norisoprenoids	9.3±0.6	10.3±0.3	0.16	10.8±0.6	11.9±0.2	0.15
Phenolic compounds (mg/L) (2014/2015)						
Caftaric acid	146.8±13	141.4±1.9	0.5	122±10.6	101.5±0.4	0.0
Catechin	5.7±0.1	5±0.2	0.0	4.7±0.2	4±0.2	0.0
Caffeic acid	0.5±0.1	0.4±0	0.1	0.3±0	0.3±0	0.3
Coutaric acid	11.8±0.6	15.4±0.1	0.0	10.5±0.9	10.8±0.1	0.6
Flavanols	5.7±0.1	5±0.2	0.0	4.7±0.2	4±0.2	0.0
Hydroxycinnamic acids	0.5±0.1	0.4±0	0.1	0.3±0	0.3±0	0.3
Tartaric esters	158.6±13.9	156.8±2	0.8	132.5±11.5	112.4±0.3	0.0
Total phenolic compounds	164.8±13	162.1±2.1	0.8	137.6±11.3	116.7±0.5	0.0
Glutathione (mg/L) (2014/2015)						
Reduced glutathione	54.1±11.8	38±4	0.09	40.3±2.5	35±3.2	0.00
Oxidised glutathione	0.9±0.2	0.2±0.1	0.00	0.4±0.1	0.9±0.1	0.00
	Press juice					
Compound	**HLcontrol**	**HL-UVB**	**p-value**	**LLcontrol**	**LL-UVB**	**p-value**
Amino acids (mg/L) (2014/2015)						
Aspartic acid	26.8±1.2	31.5±1.1	0.00	44.2±1.5	48.7±1.3	0.00
Glutamic acid	137.5±2.8	145.2±2.9	0.00	159.8±4.9	164.5±3.6	0.09
Cysteine	17.2±2.1	0±0	0.00	<LOD	<LOD	
Asparagine	7.5±0.3	7±0.2	0.01	8.7±0.5	8.8±0.2	0.45
Serine	77.9±1.7	84.8±1.8	0.00	83.4±2	88.6±2.3	0.00
Glutamine	146.9±4.4	165.6±2.9	0.00	168.5±5.4	194.2±4.7	0.00
Histidine	47.2±3.4	45.6±1	0.31	45.7±3	51.4±3.1	0.01
Glycine	5.8±0.3	5.8±0.8	0.99	5.5±0.2	4.7±0.5	0.01
Threonine	94±1.6	96.4±2.4	0.07	91.9±3.1	96.5±2.4	0.02
Arginine	521.7±22.7	534.3±10	0.24	659.7±25.3	699.7±16	0.01
Alanine	252.1±6.1	258.1±5.6	0.11	271.7±9.4	295.2±7.6	0.00
Tyrosine	25.7±1	23.4±1.2	0.00	22.2±1.7	23.5±0.9	0.12
CY2	18±1.9	22.1±6.8	0.19	17±1.3	26.2±4.3	0.00
Valine	71.1±2.5	71.1±1.4	0.99	62.1±2.4	63.7±0.9	0.16
Methionine	11.2±0.8	11.9±0.6	0.09	11±0.5	12.2±0.5	0.00
Tryptophan	13.8±0.5	13.8±1	0.96	14.2±0.8	17.5±1.1	0.00
Phenylalanine	29.9±0.7	34±1.3	0.00	26.2±1.1	30.4±1.2	0.00
Isoleucine	16.8±0.7	16.6±0.7	0.63	14±0.8	15.3±2.3	0.23
Ornithine	26.5±3.5	25.5±1.3	0.53	25.5±2.2	0±0	0.00
Leucine	26.2±1.1	25.3±1.1	0.19	21.4±0.9	22.5±0.5	0.03
Lysine	18.8±2.1	20.7±3.1	0.23	20.1±1.6	23.4±3.8	0.08
Hydroxyproline	314.7±59.8	409.9±61.9	0.02	336.6±73.2	447.5±77	0.03
Proline	409.5±43.6	508.8±46.5	0.00	386.9±28.1	421.6±7.1	0.02
GABA	211.4±7	216.6±6.5	0.21	178.5±5.6	185.8±3.9	0.03
Total aromatic amino acids	140.5±3.9	142.3±4.2	0.47	124.6±4.5	135.1±3	0.00

Total branched chain amino acids	114.1±4	113±2.8	0.59	97.5±3.9	101.5±2.5	0.07
Total amino acids	2213.4±56	2364.4±74	0.00	2338.1±62	2494.5±41	0.00
Volatile compounds (µg/L) (2014/2015)						
Eucalyptol	1.2±0	1.3±0	0.18	1.1±0.1	1.2	0.49
6-MHO	1.7±0.1	2±0	0.10	2.3±0.7	1.8±0.2	0.43
Hexanol	3.3±0	1.7±0	0.00	2±0	1.7±0.6	0.56
Linalool	27.3±0.5	14.1±0.3	0.00	6.2±0.1	6.7±2.9	0.84
α-Terpeniol	3.4±0.1	2.8±0	0.01	2.1±0	1.6±0.7	0.45
Geraniol	3.9±0	4.1±0	0.01	3.9±0	3.9±0.2	0.85
Nerol	4.2±0	4.3±0	0.10	3.6±0.1	2.6±1.2	0.38
Damascenone	5.7±0.3	7.8±0	0.01	6.5±0.2	8.8±0.7	0.04
α-Ionone	1.2±0	1.2±0	0.01	1.2±0	1.2±0	0.24
β-Ionone	1.8±0	1.8±0.1	0.96	2±0	2.5±0	0.04
Total monoterpenes	40.1±0.5	26.5±0.3	0.00	17±0.3	15.5±0.3	0.04
Total norisoprenoids	10.5±0.5	12.8±0.1	0.02	11.9±0.9	14.2±0.9	0.12
Phenolic compounds (mg/L) (2014/2015)						
Caftaric acid	1.1±0	0.7±0	0.0	2.7±0.2	1.3±0.1	0.0
Catechin	3±0.1	3.1±0.4	0.7	2.9±0.3	3.3±0.1	0.1
Caffeic acid	0.4±0	0.1±0	0.0	<LOD	<LOD	0.0
Coutaric acid	<LOD	1.1±0	0.0	<LOD	<LOD	0.0
Flavanols	3±0.1	3.1±0.4	0.7	2.9±0.3	3.3±0.1	0.1
Hydroxycinnamic acids	0.4±0	0.1±0	0.0	<LOD	<LOD	0.0
Tartaric esters	1.1±0	1.8±0	0.0	2.7±0.2	1.3±0.1	0.0
Total phenolic compounds	4.5±0.1	5±0.4	0.1	5.6±0.5	4.5±0.1	0.0
Glutathione (mg/L) (2014/2015)						
Reduced glutathione	2.5±0.1	1.7±0.1	0.00	1.7±0.1	1.4±0.1	0.09
Oxidised glutathione	2.6±0.1	4.1±0.4	0.01	4.3±0.1	4±0.3	0.00
	Settle juice					
Compound	**HLcontrol**	**HL-UVB**	**p-value**	**LLcontrol**	**LL-UVB**	**p-value**
Amino acids (mg/L) (2014/2015)						
Aspartic acid	27.5±1.1	40.1±1.9	0.00	49±1.9	64.1±5.3	0.00
Glutamic acid	135.8±1.4	171.5±4.8	0.00	185.3±10.1	181±6.9	0.53
Cysteine	<LOD	<LOD		<LOD	<LOD	
Asparagine	6.2±0.1	8.2±0.8	0.00	8.4±0.2	11.1±0.4	0.00
Serine	72.9±0.8	96.2±4.1	0.00	95.8±5.1	91.8±1.8	0.24
Glutamine	133.7±1.6	175.6±11.7	0.00	186.7±10.2	186.6±5.7	0.98
Histidine	40.7±1.5	57.8±4.6	0.00	50.5±2.9	59.4±0.2	0.00
Glycine	4±0.4	5.1±2.2	0.27	3.8±0.9	5.3±0.4	0.03
Threonine	93.1±1.3	114.2±5.8	0.00	107.5±5.8	103.8±1.1	0.33
Arginine	485.1±8.2	606.9±43	0.00	772.9±49.3	707.4±14.8	0.07
Alanine	234.9±2.9	303.6±5.5	0.00	305.9±15.4	322.4±9.2	0.14
Tyrosine	23.5±1.3	22±1.5	0.17	20.5±0.9	24.6±3.3	0.02

CY2	26.3±3.4	0±0	0.00	4.9±12.1	<LOD	0.52
Valine	67.2±0.9	73±6.4	0.05	68.4±2.3	62.7±4.9	0.04
Methionine	11.5±0.6	12.3±1	0.13	10.5±0.6	12.4±2.4	0.09
Tryptophan	11.8±0.6	9.5±1.9	0.03	12.7±0.2	9.2±0.1	0.00
Phenylalanine	29.8±0.8	34.5±2.5	0.00	27±0.5	28.3±3.7	0.38
Isoleucine	16.3±0.6	19.5±1.6	0.00	16.5±1.2	18.5±3.3	0.20
Ornithine	32.3±5.1	32.4±0	1.00	28.4±1.8	25.7±0	0.04
Leucine	25.4±0.9	29±0.5	0.00	25.3±0.8	24.3±2.6	0.39
Lysine	21.8±1.7	<LOD	0.00	<LOD	<LOD	
Hydroxyproline	373.2±50.5	98.6±28.9	0.00	521.4±108	123.1±23	0.00
Proline	460.2±24.5	543.5±114.6	0.11	450.7±15.8	545.5±104.1	0.05
GABA	201.2±3.2	250.7±18.3	0.00	218.8±13.8	190.2±5	0.01
Total aromatic amino acids	132.4±3	139.1±10.8	0.18	128.6±2	124.8±11.3	0.42
Total branched chain amino acids	108.9±2.1	121.6±8.3	0.01	110.2±3.8	105.5±10.4	0.28
Total amino acids	2161.3±5	2605.7±138	0.00	2649.7±116	2674.4±148	0.80
Volatile compounds (µg/L) (2014/2015)						
Eucalyptol	1	1.3±0	0.09	1.2	1.2	
6-MHO	2.6±0.2	2.8±0.6	0.66	1.6±0.2	2.2±0.4	0.21
Hexanol	3.1±0.4	1.9±0	0.06	1.7±0.1	1.9±0.7	0.75
Linalool	29.1±3	16.8±2.9	0.05	8.4±1.7	7.5±3.2	0.76
α-Terpeniol	4.9±0.1	3.5±0.5	0.05	1.8±0.9	1.7±0.8	0.94
Geraniol	3.9±0	4.1±0	0.04	3.9±0	4±0.3	0.82
Nerol	4.3	4.2±0.6	0.93	2.1±2	1.7±1.7	0.87
Damascenone	10.6±1.1	12.3±1.7	0.35	10.8±1	11.5±0.7	0.50
α-Ionone	1.2±0	1.1±0	0.32	1.2±0	1.1±0	0.09
β-Ionone	1.5±0.4	1.8±0.1	0.43	1.6±0.5	1.5±0.4	0.88
Total monoterpenes	42.8±4	29.8±4	0.05	14.8±4.8	15.5±0.1	0.85
Total norisoprenoids	15.9±1	18.1±1.1	0.18	15.1±0.3	16.3±1.5	0.39
Phenolic compounds (mg/L) (2014/2015)						
Caftaric acid	1±0.1	0.6±0.1	0.0	1.2±0.1	0.5±0	0.0
Catechin	3±0.3	3.2±0.1	0.2	3±0.2	3.1±0.2	0.5
Caffeic acid	0.2±0	<LOD	0.0	<LOD	<LOD	0.0
Coutaric acid	<LOD	<LOD	0.0	<LOD	<LOD	0.0
Flavanols	3±0.3	3.2±0.1	0.2	3±0.2	3.1±0.2	0.5
Hydroxycinnamic acids	0.2±0	<LOD	0.0	<LOD	<LOD	0.0
Tartaric esters	1±0.1	0.6±0.1	0.0	1.2±0.1	0.5±0	0.0
Total phenolic compounds	4.2±0.3	3.9±0.2	0.2	4.2±0.2	3.6±0.3	0.0
Glutathione (mg/L) (2014/2015)						
Reduced glutathione	2.3±0	1.6±0.1	0.00	1.7±0.2	1.5±0.1	0.00
Oxidised glutathione	2.2±0.1	3.4±0.4	0.00	3.2±0.2	3.8±0.2	0.01



Supplementary figure 5.1 Hierarchical cluster analysis of all variables from the crushed juice measured in the four microclimates.



Supplementary figure 5.2. Hierarchical cluster analysis of all variables from the pressed juice measured in the four microclimates.

Chapter 6

A description of wine composition and styles obtained from Sauvignon Blanc grapes produced in four different microclimates where light exposure and UVB levels were modulated

6.1 Introduction

Wine colour, aroma and flavour profiles, in addition to the sugar-acid balance and mouthfeel characteristics strongly contribute to the overall quality analyses of wines, and particularly when sensorially evaluated. For any specific varietal wine, these features result from the combination and interaction of a myriad of factors ranging from varietal characteristics to those which initially impact berry composition through to the winemaking procedures. The contributions of grape components to the wine characteristics have been extensively studied and numerous recent reviews exist which summarise the existing knowledge (Parker et al., 2017; Villamor and Ross, 2013; Robinson et al., 2014; González-Barreiro et al., 2015; Cosme et al., 2016; Niimi et al., 2017).

When considering the major aromatic compounds found in wine, these can be generically categorised into monoterpenes, norisoprenoids, esters, higher alcohols, fatty acids, pyrazines and volatile sulphur compounds. Many of these compounds can be directly related to the volatile compound and amino acid composition of the grape berries at harvest. Of further significance are the microbiological components and their contributions and stability during winemaking and aging. The complex interactions between yeast, fungi and bacteria present in the natural grape microbiome and those which are purposefully added during winemaking create the subtle nuances and uniqueness found in wine flavour responses (Fleet, 2003; Liu et al., 2017). The multifarious relationships between the diverse chemical compounds and microorganisms present during wine processing and storage therefore ultimately determine both the chemical profiles and the sensory perception of the final wines.

Alcoholic wine fermentation is mostly driven by *Saccharomyces cerevisiae*, however the consortium of other yeasts and bacteria present and the interactions between them will influence the composition and the progression of fermentation. The grape berry essentially provides the substrates necessary for fermentation, including nitrogen-containing compounds, which may yield various yeast (fermentation)-derived aroma compounds. Amino acids make a portion of the yeast assimilable nitrogen (YAN) which is necessary for yeast growth and development. The metabolism of certain of these amino acids, namely the branched chain (BCAA) and aromatic amino acids (AA) will result in the formation of important volatile compounds including higher alcohols, volatile fatty acids and esters which would all contribute to the final wine sensorial profile (Hazelwood et al., 2008; Swiegers et al., 2005). The amino acid composition of the juice prior to fermentation will therefore influence the wine aromatic profiles.

The grape-derived volatile compounds are dependent on the specific variety, the disease status, environmental impacts and management practices, amongst others. The volatile compounds impart diverse aromatic characteristics including floral, fruity and herbaceous-like aromas on the final products. Known compound groups that contribute to the varietal characters of the wines are terpenes, carotenoid-derived norisoprenoids, C_6-compounds, green leaf volatiles (GLV), methoxypyrazines and the volatile thiol precursors. Sauvignon Blanc is one of the best characterized varietals in terms of the odour-active compounds that define the wine styles from this international cultivar. It is known for its distinctive aromas which range from herbaceous and vegetative to intensely tropical. These flavour profiles can be attributed to a number of compounds present in the wine, including volatile thiols, methoxypyrazines and yeast derived compounds such as esters and fatty acids (Coetzee and du Toit, 2012, 2015).

Amongst the many factors that could influence the grape composition, the microclimate of the bunch zone has a considerable effect on berry development and ripening and therefore exercises a significant influence on the berry flavour and aroma potential. These characteristics are then essentially reflected to a degree in the wine. In Sauvignon Blanc, leaf removal is a commonly practiced viticultural treatment leading to a consequent increase in light incidence in the bunch zone. Higher light exposure has been shown to reduce the green characteristics of the wine and enhance the more tropical and fruity flavours and aromas. This has mostly been explained by the inherent sensitivity of methoxypyrazines to light and their consequent degradation with increased light exposure (Gregan et al., 2012; Martin et al., 2016; Sivilotti et al., 2017; Suklje et al., 2014). The green aromas including “green pepper,” “asparagus” and “grassy” are usually related to the methoxypyrazines, however other compounds including C6’s and other GLVs may also contribute to these characters. These compounds are produced via the lipoxygenase-hydroperoxide lyase (LOX-HPL) pathways and are developmentally regulated and have also been shown to be released during damage such as is incurred during mechanical harvesting (Herbst-Johnstone et al., 2013) and maceration (Olejar et al., 2015b).

The volatile thiols constitute an important group of odour impact compounds in Sauvignon Blanc and contribute to the tropical, grapefruit, passionfruit and guava aromas. Typically, they are not present in the grape berries or juice, but are generated by the yeast during alcoholic fermentation from non-volatile precursors found in the berry. A few trials have shown higher thiol concentrations in wines made from exposed grapes (Gregan et al., 2012; Sivilotti et al., 2017; Suklje et al., 2014), however the associated increase in tropical attributes in these wines was interpreted to be likely more related to lowered levels of methoxypyrazine (sensory perception threshold in wine of 2 ng/L) and the consequent suppression of its masking effect, thereby allowing for the expression and perception of the more fruity attributes (Suklje et al., 2014).

Phenolic development and composition in the grape berries may also be modified by external environmental factors and viticultural practices. Increased or reduced light exposure to the grape

bunches represents an important and influential factor in phenolic compound development and UVB attenuation has been shown to reduce their accumulation in ripe berries (Koyama et al., 2012a). In white wines such as Sauvignon Blanc, it is accepted that skin contact should not be extended due to concerns regarding increased phenolic extraction which could lead to bitterness and astringency, specifically since the phenolic profile not only varies in composition, but also content in the different subcellular locations of the berry. Extraction of these compounds from skins and seeds have however been shown to contribute to the antioxidant activity of white wines which in turn could reduce the effects of juice oxidation and lead to the preservation of varietal thiols (Coetzee and du Toit, 2015; Kilmartin et al., 2015; Olejar et al., 2015a). Mechanical harvesting has also been shown to lead to increased phenolic content and therefore antioxidant activity (Olejar et al., 2015b).

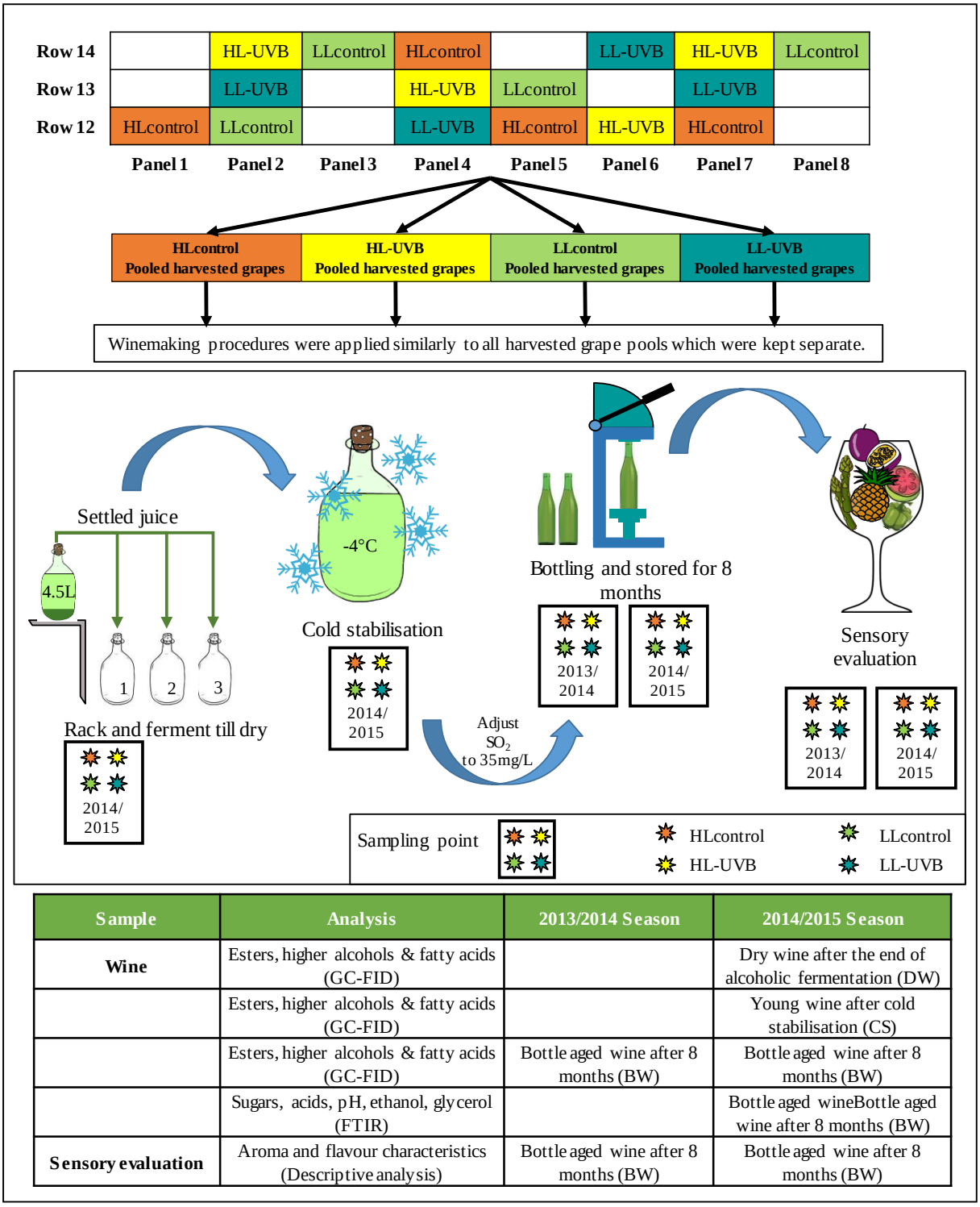
The links between grape and wine composition and particularly the ability to understand and predict the outcomes of viticultural treatments on the final products remain an important field of study. The overall aim of this work was to study the impact of light exposure from both the perspectives of quantity and quality (specifically focussing on UVB attenuation) of light on Sauvignon Blanc in a grapes-to-wine analysis. The characterisation of UVB effects on berry development was achieved in a multi-season field experiment where microclimatic scenarios were established in a Sauvignon Blanc vineyard that allowed to evaluate UVB attenuation impacts in both a high and low light environment (Chapters 3 and 4). The experiment was further extended to follow the typical wine-making procedure and generated a further characterisation of the juice processing stages (Chapter 5, for a characterisation of the UV impacts on the juices before alcoholic fermentation). Here the wines made from the four microclimates are being described and compared based on chemical analyses that focused on the concentrations of esters, higher alcohols and fatty acids present. These wines created from the four different juices were analysed for mainly fermentation-derived volatile compounds and the final wine subjected to sensory analysis to elucidate the effects of UVB in HL and LL microclimates.

6.2 Materials and Methods

6.2.1 Wine making and overview of analysis over two seasons

Wines were made from the grapes from the four individual microclimates for two consecutive seasons. In both years, the harvesting of the grapes occurred in conjunction with the commercial harvest for that specific vineyard block at around 21/22°B. All panels were harvested on the same day, but the two controls and two treatments were harvested and processed separately. This is described in more detail in Chapter 5. The four individual juices were allowed to clarify overnight at 4ºC, after which each control/treatment was divided into three equal volumes to represent three fermentation repeats. Dry ice and CO_2 were used throughout the process to prevent oxidation. The juice was inoculated with Lalvin QA23 (Lallemand, Canada) and fermented at 15°C until dry (residual sugar <5 g/l). This was monitored

with a hydrometer for all 12 fermentations. FTIR analysis was also conducted on the wine at the end of alcoholic fermentation in the 2014/2015 season. No additions other than SO_2 were made to the juice or wine at any stage during processing or fermentation, including bentonite or DAP. The resulting wines were cold stabilised at 4°C for four days, after which the free SO_2 was adjusted to 35 mg/l. Wines were bottled and stored at 15°C until sensory evaluation which took place 8 months after bottling Figure 6.1 depicts the general outline of the experimental layout in the vineyard and the process used to make the wine, thereby placing the wine in context of the entire experiment, linking it back to the vineyard treatments and the harvest and sampling strategies.



Sample	Analysis	2013/2014 Season	2014/2015 Season
Wine	Esters, higher alcohols & fatty acids (GC-FID)		Dry wine after the end of alcoholic fermentation (DW)
	Esters, higher alcohols & fatty acids (GC-FID)		Young wine after cold stabilisation (CS)
	Esters, higher alcohols & fatty acids (GC-FID)	Bottle aged wine after 8 months (BW)	Bottle aged wine after 8 months (BW)
	Sugars, acids, pH, ethanol, glycerol (FTIR)		Bottle aged wineBottle aged wine after 8 months (BW)
Sensory evaluation	Aroma and flavour characteristics (Descriptive analysis)	Bottle aged wine after 8 months (BW)	Bottle aged wine after 8 months (BW)

Figure 6.1. The winemaking procedure employed during this experiment in both seasons. The sampling points are indicated on the figure for both seasons. Each of the coloured stars represent a different microclimate as is indicated in the figure key. The accompanying table indicates which samples were taken and what analysis was done in in each season.

6.2.2 Determining general oenological wine characteristics

The four dry wines were evaluated in the 2014/2015 season for fructose, glucose, total sugars, ethanol, malic acid, pH, volatile acids, glycerol and titratable acidity (TA) with Fourier transform mid-infrared spectroscopy (FT-MIR; WineScan F120, FOSS Analytical, Denmark) according to the method described by Louw et al. (2009).

6.2.3 Fermentation derived major volatile compound analysis

Following the winemaking process, wine samples were analysed for the fermentation derived major volatile aroma compounds using gas chromatography with flame ionized detection (GC-FID) as validated in Louw et al. (2009). The analysis was conducted at the time points outlined in Figure 6.1. At each time point, samples were taken from the four individual treatments, each of which had been split into three fermentation repeats. Three samples were therefore provided from each treatment, amounting to 12 samples in total per time point.

The volatile fraction of each sample was extracted by adding 1ml diethyl ether to 5 ml of wine with an internal standard, namely 4-Methyl-2-Pentanol (100 µl of 0.5 mg/l solution in a soaking solution). This mixture was placed in an ultrasonic bath for 5 minutes and then centrifuged. Anhydrous sodium sulphate was used to dry the ether layer which was removed after centrifugation. The resulting extract was then injected into the GC-FID (Hewlett Packard 6890 series GC system). Compounds were then extracted according to the method described in (Louw et al., 2009).

Each sample was extracted three times and then injected twice. The HP Chemstation software was utilised for manual data collection and for the integration of the resulting peaks. The resulting data sets were statistically evaluated using Excel (Microsoft Office Professional Plus 2016), Statistica (version 12) and SIMCA **(**Sartorius Stedim Data Analytics AB**).**

6.2.4 Wine sensory analysis

Descriptive analysis was done on the wines following 8 months in the bottle for both experimental seasons using a trained panel (Figure 6.1). The panel in both years consisted of experienced Sauvignon Blanc sensory assessors (8 females and 1 male) of variable age ranging from 25 to 55. Training comprised four 2 hour training sessions. Initially, the panel generated a list of appropriate descriptors for the four wines which were narrowed down to the most prominent in the following training sessions. Reference standards were subsequently made up for the selected descriptors and the panel trained to discriminate between them. This was followed by intensity scaling during which the selected attributes were scaled according to their perceived intensity in the wines. The scale ranged to 0 to 100, with zero representing “none” and 100, “very high intensity.” On completion of the training sessions, the wines

were assigned a randomised 3 digit code based on the William design Latin-square and presented to the panel for evaluation. Each fermentation repeat for each wine was evaluated in triplicate. Black glasses were used for tasting to exclude the differences in colour and the evaluations were conducted in separate tasting booths in a controlled, well ventilated sensory lab. The resulting data sets were evaluated statistically using PanelCheck (www.panelcheck.com, Nofima), SIMCA and Excel.

6.3 Results

6.3.1 Basic wine analysis conducted on the dry wine at the end of alcoholic fermentation

The four different wines all fermented to dryness within seven days in both seasons. No obvious differences were observed in terms of fermentation speed and basic oenological measurements (Table 6.1). The dry wine revealed similar pH, glycerol and volatile acids, whereas small differences were recorded in residual sugars and acid levels. The HLcontrol environment yielded wines with the highest residual sugar levels, but lowest malic acid and TA, whereas UVB attenuation in the LL environment yielded a wine with the lowest residual sugar, highest malic acid and TA. The fructose: glucose ratios for the four wines are indicated in Table 6.1 with the greatest ratio seen in the HLcontrol wine.

Table 6.1. The FTIR results showing the basic measured oenological parameters in the four individual dry wines (2014/2015 season). Three biological repeats and two technical repeats per biological repeat were used. Values are listed as the mean ± standard deviation. Letters indicate significant differences.

	Fructose	Glucose	Total sugar	Fru:Glu	Ethanol	Malic acid	pH	Volatile acid	Glycerol	TA
HLcontrol	4±0.4^{a}	0.7±0.1^{a}	4.7±0.3^{a}	5.7^{a}	13.4±0.1^{a}	3.2±0^{a}	3.1±0^{a}	0.4±0^{a}	6.6±0.2^{a}	7.8±0^{a}
HL-UVB	2.6±0.3^{b}	0.8±0.1^{a}	3.5±0.3^{b}	3.3^{b}	13.6±0.1^{a}	3.2±0.1^{a}	3.1±0^{a}	0.4±0^{a}	6.5±0.2^{a}	8.1±0^{b}
LLcontrol	1.4±0.2^{c}	0.6±0.1^{b}	2±0.3^{c}	2.3^{b}	12.7±0^{b}	4.1±0.1^{b}	3.1±0^{a}	0.4±0^{a}	6.3±0.1^{a}	8.6±0^{c}
LL-UVB	1.2±0.2^{c}	0.5±0.2^{b}	1.7±0.3^{c}	2.4^{b}	12.3±0^{b}	4.3±0.1^{b}	3±0^{a}	0.4±0^{a}	5.8±0.1^{b}	8.9±0.1^{d}

6.3.2 Chemical analysis of wine samples

The chemical profiles were generated with a GC-FID method that profile and quantify the major volatiles covering the following compound groups: esters, higher alcohols and fatty acids. Initially the analysis was only conducted on samples after the wines were aged in the bottles for 8 months, coinciding with the sensory evaluation of the wines (for the 2013/2014 season). In the subsequent season (2014/2015), samples were analysed from wines at the end of alcoholic fermentation, after cold stabilisation and after the bottle ageing period. The full dataset is provided in Supplementary Table 6.1.

To identify sample profiles with similar features over the different wine stages, the volatile data of the 2013/2014 (season 1) and 2014/2015 (season 2) years were subjected to hierarchical clustering (Figure 6.2 A). The BW (of which data was available for two seasons) clustered separately from the young wines (DW and CS wine that were analysed only in one of the seasons). Within the young wine cluster, samples grouped according to light exposure whereas UVB attenuation defined smaller clusters within these HL and LL groups. In the bottle aged wine, for both seasons, the HL and LL exposure samples generally clustered together while the UVB impacts became more subtle. The associated cluster expression matrix and mean patterns identified compounds of similar response patterns (Figure 6.2 B). In the 2014/2015 season samples, where three processing steps were evaluated, it was clear that certain compounds displayed a clear pattern of either diminishing, accumulating or being stable over the different wine samples, grouping correlating compounds into each cluster. Furthermore, it was revealed that while certain compounds showed different expression patterns between the two seasons in the BW, a number of the measured volatiles were similar. It is clear from the data that different compound clusters responded differentially to wine stage and light exposure, whereas the effects of UVB attenuation were subtle.

Cluster 2 demonstrated a downward trend in compound concentrations, with levels higher in the young wines when compared to the bottle aged wine samples. Compounds in this cluster included important aromatic volatiles such as isoamyl acetate, isoamyl alcohol and ethyl phenylacetate. Cluster 3 also emphasised the difference between the young and aged wine samples, with levels in the bottle aged wines being considerably higher. These compounds included the ethyl ester pool as well as ethyl caprylate, ethyl caprate and and hexanol, all of which impart specific aromatic characteristics.

Compounds in clusters 4 and 5 showed UVB responses in the HL and LL samples (i.e. irrespective of light microclimate of the grapes). Cluster 4 contained mainly esters, but also the total fatty acid pool and showed higher levels in the HLcontrol wine after then end of alcoholic fermentation. Cluster 5 contained mostly higher alcohols, but also the total ester and higher alcohol pool. These were seen to be higher in the LL microclimate wines, however in the bottle aged wine, HL-UVB samples exhibited similar levels to the LL wines, while the HLcontrol wine levels remained low.

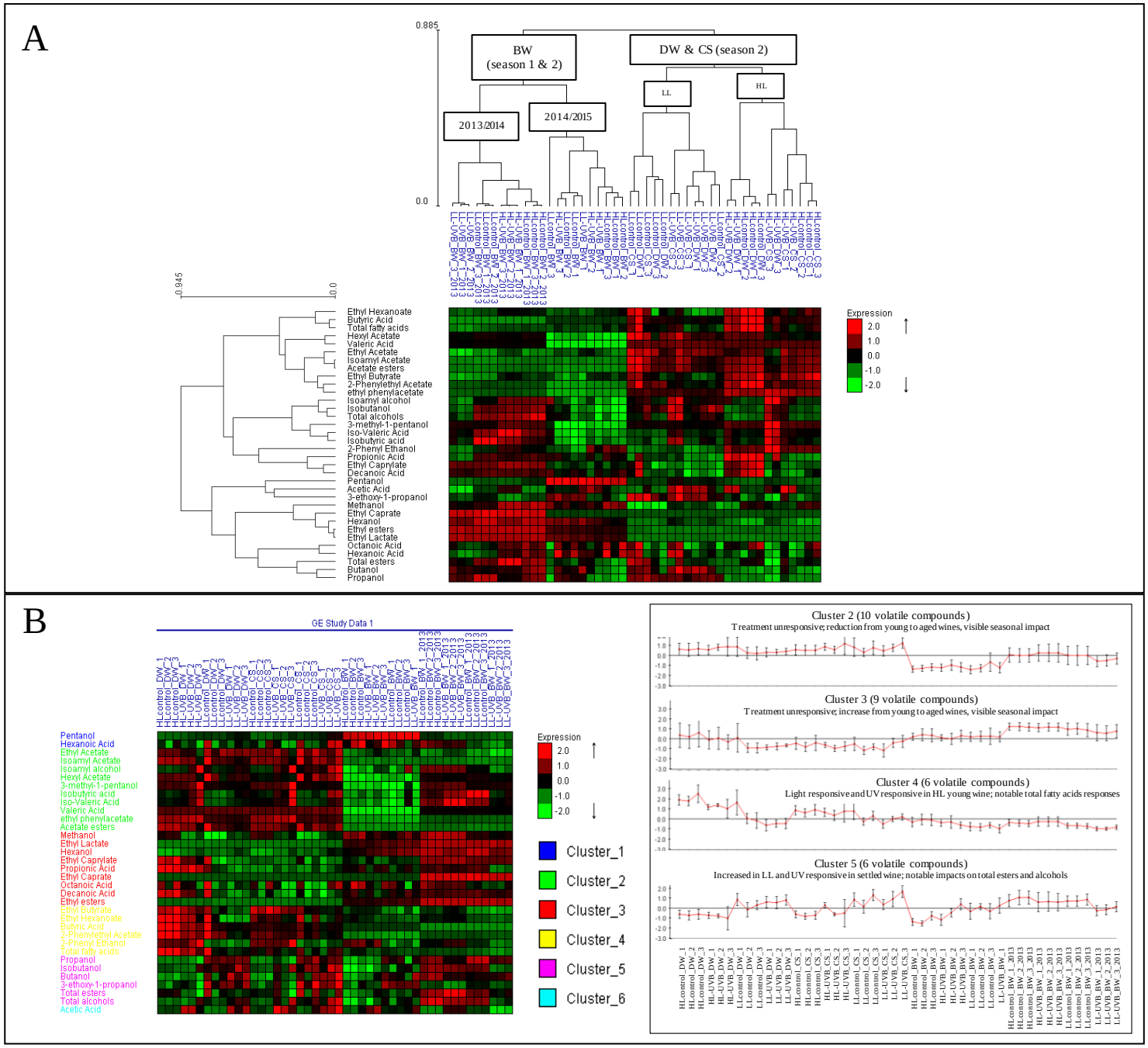


Figure 6.2. Hierarchical clustering of volatile compound data for all four treatments in the three different wines for both seasons (A). The associated cluster expression matrix identifies compounds of similar response patterns. The most important mean clustering patterns are presented (B).

It is clear that significant differences are present between the different wine stages, most notably between the young wines (DW & CS) and aged wines (BW). To better elucidate the most significant responses which occurred between the early wines and aged wines, the data from the 2014/2015 season was further statistically investigated and validated using repeated measures ANOVA in order to rank the significance of each compound in response to the three main experimental factors (i.e. wine stage, light exposure and UVB attenuation) individually, and in combination (Table 6.2). Each of the compound groups (esters, higher alcohols and fatty acids) is presented separately. The results confirmed that wine stage was the most significant driver in the dataset in each of the compound groups, followed by the variation in light exposure. As shown in the clustering analysis, UVB attenuation impacts were very subtle, being blurred by the stronger impacts of light exposure and wine stage. A few compounds from each group were seen to respond to the attenuation of UVB including 2-phenylethyl acetate, ethyl hexanoate and butyric acid, all of which were found in cluster 4 (Figure 6.2). More UVB responsive compounds were however seen in combination with wine stage, including a few of the esters and fatty acids. The level of significance was however low in all cases, except in 2-phenylethyl acetate which was the most responsive. Ethyl phenylacetate and methanol were responsive to UVB attenuation in combination with light exposure.

Table 6.2. An analysis of the major volatile compounds in the different wines (2014/2015 season). The repeated measures ANOVA results for the listed parameters and individual compounds are reported as F-values. Values are scaled from highest (i.e. most significant) to lowest by colour. Green indicates low F-values, while red indicates high F-values values. All insignificant values (F≤3) are coloured in grey. Different superscripted letters indicate significant differences between variables: p-value < 0.001 [a]; 0.001< p-value < 0.01[b]; 0.01 < p-value < 0.05[c] and insignificant [d] Maximum ■ ; 50% ■ ; minimum ■ ; insignificant ■

	Wine stage	Exposure	UVB-attenuation	Exposure x Wine stage	UVB-attenuation x Wine stage	UVB-attenuation x Exposure	UVB-attenuation x Exposure x Wine stage
Esters							
Ethyl Lactate	1110.6[a]	13.3[b]	3.0[d]	4.3[c]	0.2[d]	0.0[d]	1.0[d]
Ethyl phenylacetate	937.0[a]	229.3[a]	3.6[d]	23.2[a]	1.4[d]	12.5[b]	2.0[d]
Ethyl esters	849.3[a]	0.6[d]	0.2[d]	7.1[b]	0.1[d]	0.0[d]	1.2[d]
2-Phenylethyl Acetate	190.1[a]	14.8[a]	18.4[a]	11.0[a]	16.7[a]	0.2[d]	0.6[d]
Ethyl Caprate	165.8[a]	2.4[d]	0.3[d]	2.4[d]	3.5[d]	2.5[d]	1.1[d]
Acetate esters	118.5[a]	0.3[d]	3.2[d]	3.6[d]	4.4[c]	0.0[d]	0.3[d]
Isoamyl Acetate	78.4[a]	0.0[d]	2.9[d]	2.8[d]	3.6[c]	0.1[d]	0.4[d]
Hexyl Acetate	51.1[a]	5.9[c]	0.0[d]	4.1[c]	1.5[d]	0.0[d]	0.2[d]
Ethyl Acetate	47.0[a]	4.3[d]	0.0[d]	1.1[d]	6.0[b]	0.4[d]	1.4[d]
Ethyl Butyrate	27.9[a]	21.6[a]	4.9[d]	1.7[d]	1.0[d]	0.2[d]	0.2[d]
Ethyl Caprylate	8.6[a]	4.0[d]	2.5[d]	9.5[a]	1.0[d]	0.1[d]	0.4[d]
Ethyl Hexanoate	4.8[c]	7.0[c]	5.1[c]	6.7[b]	1.9[d]	0.0[d]	0.1[d]
Total esters	0.9[d]	2.5[d]	0.5[d]	0.1[d]	4.4[c]	0.3[d]	0.8[d]
Higher alcohols							
Pentanol	1023.5[a]	40.4[a]	25.6[a]	1.6[d]	1.7[d]	1.1[d]	0.2[d]
Hexanol	474.6[a]	21.9[a]	20.6[a]	5.4[c]	1.2[d]	0.4[d]	1.1[d]
Methanol	80.3[a]	49.7[a]	0.1[d]	7.3[b]	6.5[b]	70.0[a]	15.3[a]
3-methyl-1-pentanol	68.3[a]	8.1[c]	0.1[d]	1.0[d]	1.5[d]	3.5[d]	0.0[d]
Isobutanol	24.6[a]	10.0[b]	2.3[d]	0.2[d]	1.0[d]	0.1[d]	1.8[d]
Isoamyl alcohol	11.3[a]	0.4[d]	4.0[d]	0.7[d]	1.9[d]	1.4[d]	0.0[d]
Total alcohols	9.0[a]	4.2[d]	3.8[d]	0.2[d]	1.0[d]	0.0[d]	0.7[d]
2-Phenyl Ethanol	1.6[d]	44.9[a]	0.1[d]	0.5[d]	1.3[d]	0.8[d]	0.5[d]
Propanol	1.4[d]	63.6[a]	0.5[d]	2.3[d]	1.6[d]	0.1[d]	0.3[d]
3-ethoxy-1-propanol	0.9[d]	24.8[a]	0.2[d]	1.1[d]	0.3[d]	0.2[d]	0.1[d]
Butanol	0.9[d]	48.5[a]	19.9[a]	0.5[d]	0.8[d]	1.4[d]	0.3[d]
Fatty acids							
Valeric Acid	2386.5[a]	7.0[c]	1.9[d]	2.3[d]	0.3[d]	0.0[d]	0.0[d]
Propionic Acid	44.5[a]	29.5[a]	1.1[d]	31.9[a]	3.8[c]	1.4[d]	0.7[d]
Butyric Acid	34.2[a]	5.5[c]	5.4[c]	8.1[b]	5.3[c]	0.1[d]	0.5[d]
Iso-Valeric Acid	19.2[a]	4.9[d]	3.0[d]	1.0[d]	2.4[d]	0.2[d]	0.1[d]
Total fatty acids	18.2[a]	2.5[d]	2.0[d]	7.7[b]	4.3[c]	0.3[d]	0.0[d]
Decanoic Acid	16.2[a]	1.7[d]	4.4[d]	6.7[b]	2.8[d]	0.2[d]	0.1[d]
Isobutyric acid	12.6[a]	1.5[d]	4.5[d]	0.6[d]	2.7[d]	0.0[d]	0.1[d]
Acetic Acid	4.5[c]	0.0[d]	29.5[a]	1.0[d]	3.7[c]	0.0[d]	0.5[d]
Hexanoic Acid	3.6[c]	0.5[d]	0.0[d]	0.8[d]	0.5[d]	0.8[d]	1.2[d]
Octanoic Acid	1.4[d]	0.1[d]	0.2[d]	0.6[d]	0.9[d]	1.3[d]	1.0[d]

To better elucidate the potential effects of UVB, each HL and LL wine was also investigated separately using OPLS-DA models and ANOVA validated by Fisher LSD post hoc tests in the dry wine after the end of alcoholic fermentation (DW), the wine after cold stabilisation (CS) and the bottle aged wine after 8 months (BW). The results of the various statistical tests were summarised and are presented in a Venn diagram (Figure 6.3); considerably more compounds responded to UVB attenuation in the young wines (DW & CS) versus the bottled aged wine in both the HL and LL microclimate. The format allows to identify compounds that were seen to respond in both the HL and LL microclimate, compared to others that responded uniquely to each light exposure. A number of these belonged to the ester compound group with a few fatty acids being identified as well. Importantly, many of these compounds are involved in amino acid metabolism, being synthesised via the Ehrlich pathway from the branched chain amino acids valine, leucine and isoleucine, as well as phenylalanine. Furthermore, in the dry wine, methanol and ethyl phenylacetate showed opposite response to UVB attenuation depending on light exposure.

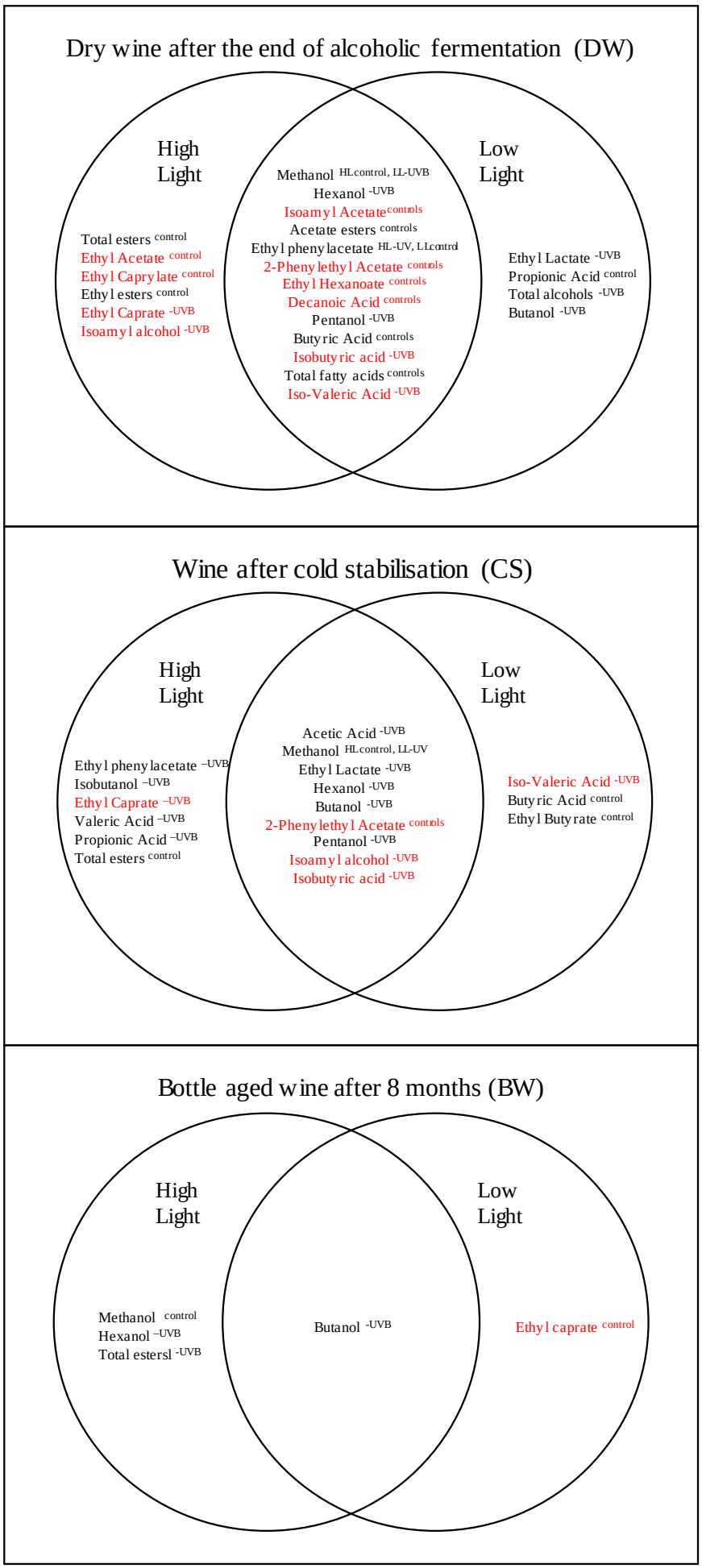


Figure 6.3. A Venn diagram showing the compounds which responded to UVB attenuation in HL and LL microclimates for the dry wine (A), cold stabilised wine (B), and bottle aged wine (C). Compounds were selected based on VIP scores for OPLS-DA models (>0.95) and on significance tested with factorial ANOVA and Fisher LSD Post Hoc tests (adjusted p-value, q-value ≤0.05). Important compounds are listed in red. The superscripted "control" and "-UVB" indicate in which treatment the specific compounds were statistically increased. In the intersections "control" indicates a significant increase in the HL and LL controls, similarly "-UVB" indicates a significant increase in the HL-UVB and LL-UVB treatments. In cases where treatment responses are differential for the HL and LL microclimate, the full treatment names are indicated.

ANOVA plots are presented in Figure 6.4 for the statistically significant UVB-responsive esters, fatty acids and alcohols which are formed from the branched chain amino acids and phenylalanine during alcoholic fermentation (indicated in red on Fig 6.3).

In the CS wine, isoamyl alcohol and iso-valeric acid showed similar responses, with UVB attenuation increasing levels, however the opposite response was seen in isoamyl acetate. Isobutyric acid was significantly increased with UVB attenuation for both microclimates in the two young wine samples. The phenylalanine derived compounds also showed different patterns depending on UVB, with ambient UVB increasing levels of phenyl ethylacetate in both the HL and LL microclimate. A significant influence of light exposure was seen in the phenyl ethanol, a trend which was maintained in all three wine processing stages. UVB attenuation led to higher levels of this compound in the HL microclimate.

Fatty acid metabolism is related to valine and isoleucine and certain associated compounds were seen to be UVB responsive. Decanoic acid was increased in both the HL and LL controls compared to the UVB attenuation treatments. UVB attenuation also reduced the levels of ethyl hexanoate and ethyl caprylate in both the HL and LL microclimates. Ethyl caprate was the only ester seen to increase during aging and was highest in the LLcontrol wine.

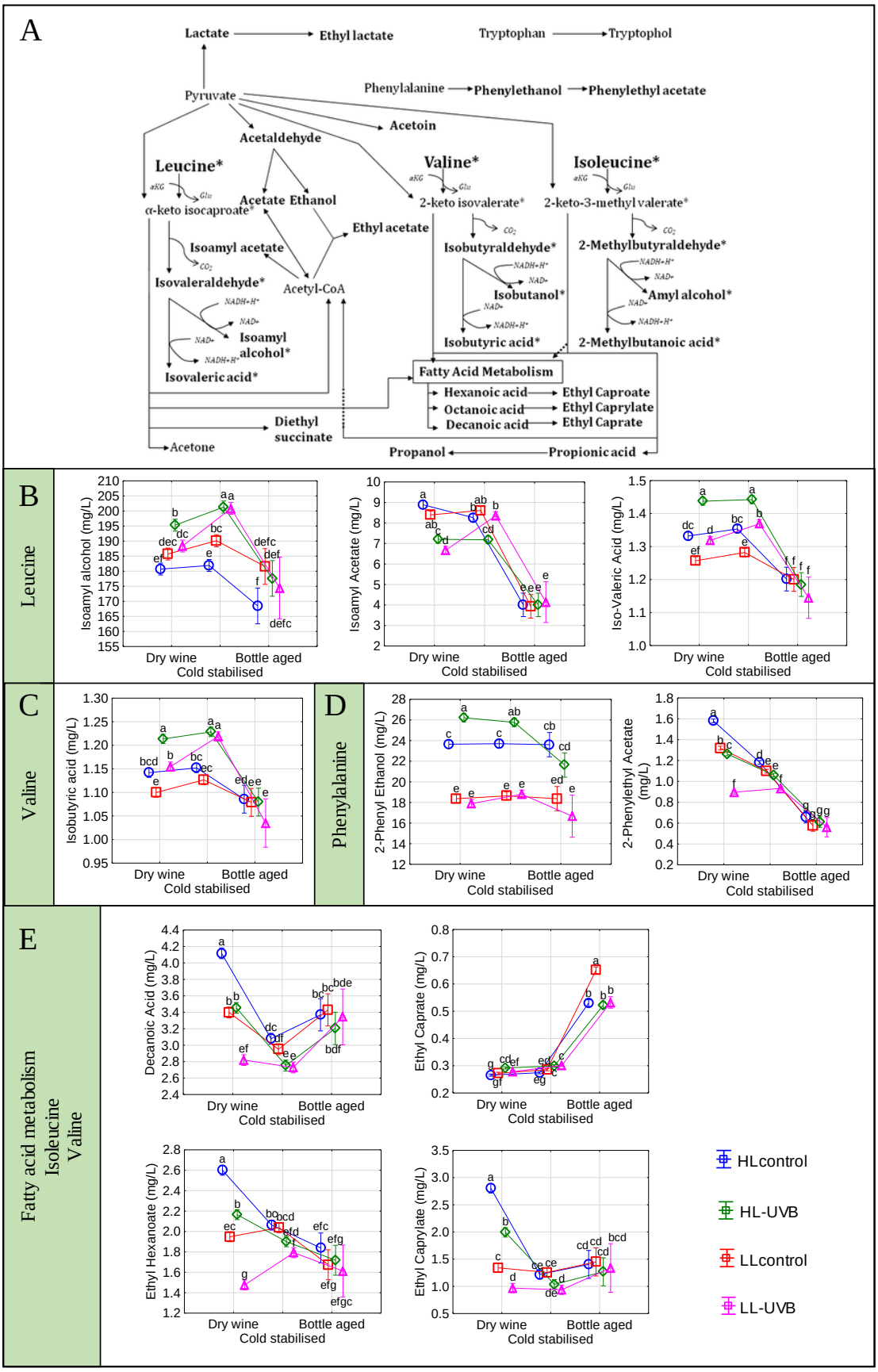


Figure 6.4. The UVB-responsive volatile compounds related to amino acid metabolism during fermentation. The Ehrlich Pathway utilises certain amino acids to form several volatile compounds (Styger et al., 2011) (A). Amino acids metabolised in this pathway include leucine (B), valine (C & D), phenylalanine (D) and isoleucine (E) to form the highlighted esters, higher alcohols and fatty acids.

6.3.3 Descriptive Sensory Analyses of bottle aged wines from two seasons

Descriptive analysis was conducted on the bottled aged wines after 8 months in both seasons. The panel performance was tested using Panel Check software and the workflow as described by Tomic et al. (2010) was used. Panel repeatability, consensus and discriminability were tested and it was found that all judges were repeatable and could discriminate between samples; the panel consensus was therefore acceptable and no judges' scores were removed (results not shown). The significance of each attribute was determined and is presented in Supplementary Figure 6.1. From these results it could be determined that in both seasons, the majority of attributes were highly significant.

Although slightly different descriptors were given by the panels of the different years, the majority of descriptors related to terms associated to typical Sauvignon Blanc styles such as "tropical" or "green." In the second season (2014/2015), additional descriptors such as "hay," "dried fruit," and "dusty" were included which did not occur in the analysis from the 2013/2014 season (Table 6.3).

Table 6.3. A list of descriptors used in the descriptive analysis of the wines in the two seasons.

Season 2013/2014	Season 2014/2015
Green beans	Grapefruit
Grapefruit	Pineapple
Pineapple	Passion fruit
Guava	Guava
Green pepper	Banana
Lemon	Peach
Asparagus	Gooseberry
Passion fruit	Asparagus
Litchi	Green pepper
Cut grass	Yellow apple
Yellow apple	Dried fruit
Banana	Tea
	Hay
	Cut grass

The descriptive analysis data is presented using biplots and are presented per season, as well as in combination. These plots showed clear treatment differences in both seasons (2013/2014 and 2014/2015) (Figure 6.5 A and B), grouping the HLcontrol and LLcontrol samples consistently separately from each other as well as the UVB attenuation samples. Unfortunately the LL-UVB sample set in Figure 6.5B is only represented by a single wine sample due to technical problems with this sample set during the sensory evaluation of the 2014/2015 season.

In both seasons, the data firstly showed an association of tropical, fruity and sweet attributes with the HL microclimate, while the LL microclimate exhibited more vegetative and grassy characteristics. Moreover, the HLcontrol wines were consistently (over seasons) more strongly associated with tropical fruity attributes than the HL-UVB wines. Regarding the LL microclimate wines, in the first season the LLcontrol and HL-UVB wines were seen to group very closely together, while the LL-UVB wines were associated with the green pepper, asparagus and canned green beans aromas. This wine was also described as being bitter and sour. In the second season both LL microclimates exhibited aromas of asparagus and green pepper, but the LLcontrol was more associated with the fresh vegetative aroma of "grassy," while the LL-UVB was shown to be more sour and astringent.

Despite the fact that vintage-specific differences were observed between the two seasons (Figure 6.5C), the major distinctive characteristic from the four microclimates were still consistently observed in the sensory evaluations.

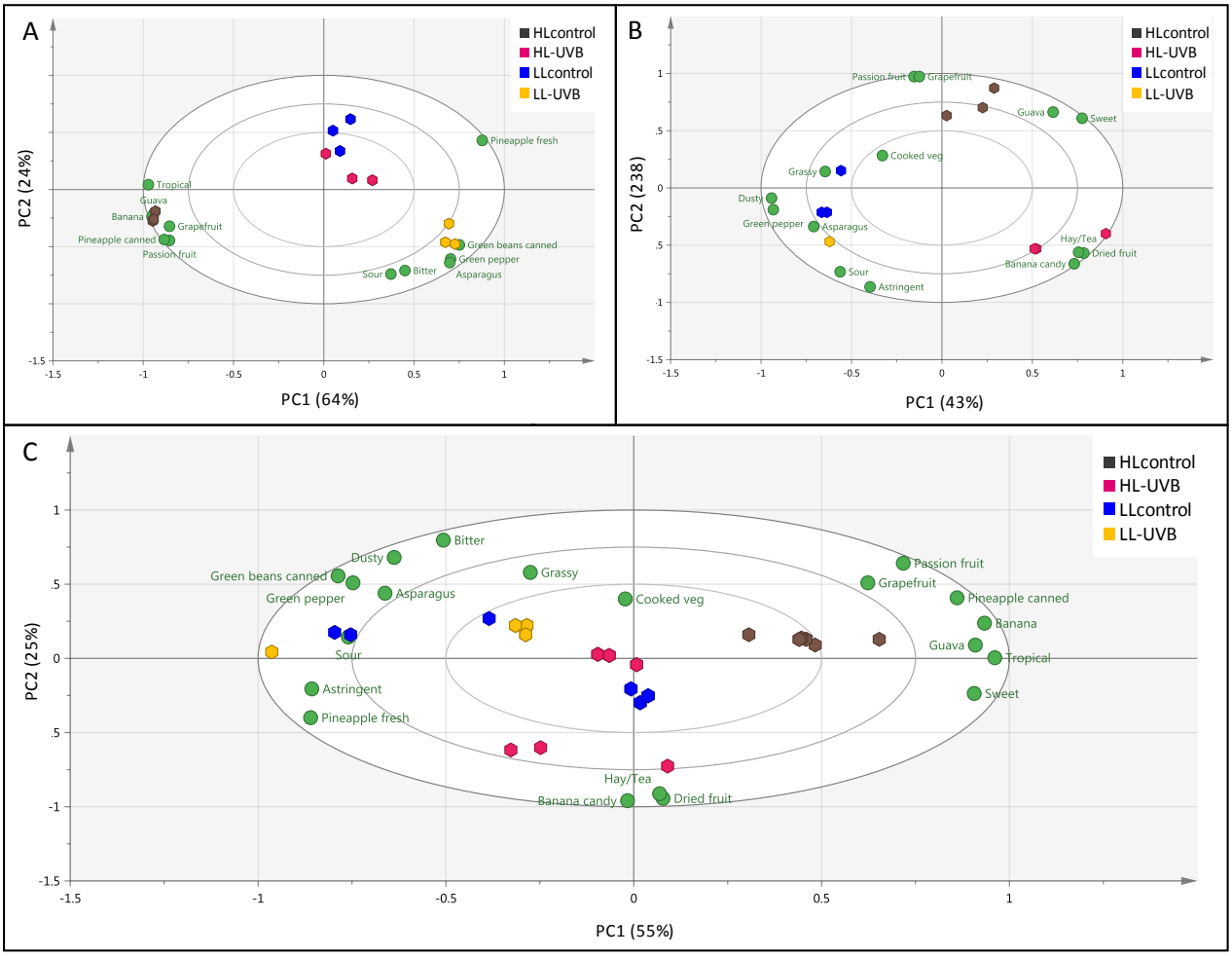


Figure 6.5. Biplots constructed from the sensory data for the four different microclimate wines for the 2013/2014 season (A), 2014/2015 season (B) and in combination (C).

6.3.4 Seasonal comparison of chemical and sensory data integrated

Since chemical and sensory analysis was conducted on two seasons of bottle aged wines, the data was also integrated into a single PCA analysis (Figure 6.6A), with two PC's explaining 43% (season) and 20% (light exposure) of the variance The corresponding loading plot indicated how measured volatile compounds and sensory attributes contributed to the model (Figure 6.6 B). The model revealed a vintage effect with more esters, higher alcohols and fatty acids associating with the 2013/2014 season. However within each year, a clear light exposure response was seen, although slightly different per year. Most notable, the "green" descriptors associated more with the LL microclimate, while the more "fruity" attributes were related to the HL microclimate. Certain compounds including ethyl butyrate, ethyl hexanoate and isoamyl acetate were also clearly higher in the HL microclimate. All of these esters impart fruity aromas such as pineapple and banana. Furthermore, within each light environment, UVB attenuation samples formed separate groupings, confirming that regardless of vintage, the treatment responses behaved similarly over the two seasons when considering both the chemical and sensory profiles of the bottle aged wines.

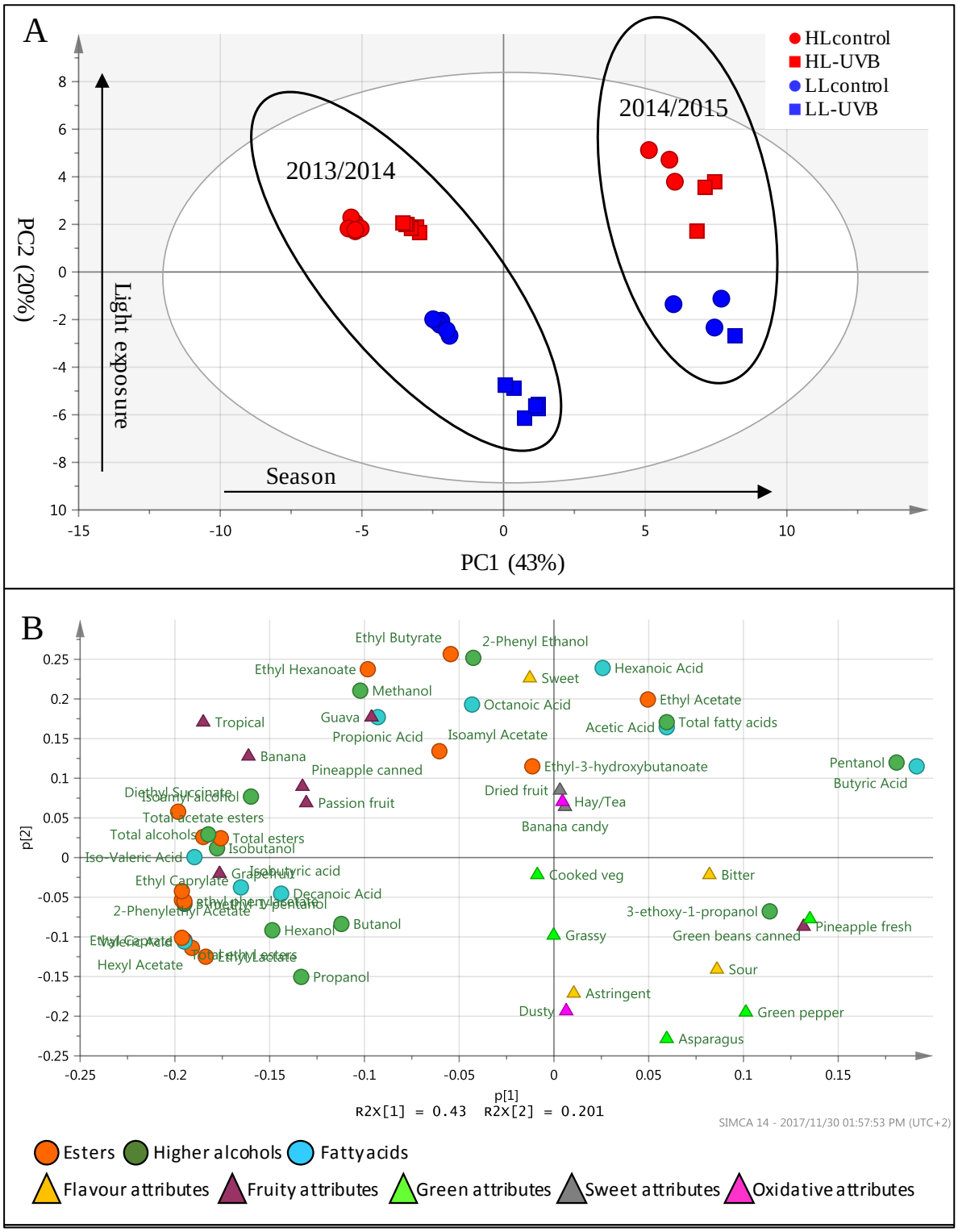


Figure 6.6. A PCA model for chemical and sensory data measured in two seasons for all four microclimates. Red symbols indicate the HL samples (HLcontrol & HL-UVB), while the blue symbols represented the LL samples (LLcontrol & LL-UVB) (A). The corresponding loading plot (B) lists the volatile compounds measured and sensory attributes. The circles represent the volatile compounds while the triangles represent the sensory attributes. Different colours represent certain compound or attribute groups.

6.4 Discussion

The results presented in this chapter forms part of a field experiment on Sauvignon Blanc in a cool climate area and here the impacts of four distinct microclimates on some targeted metabolites as well as the sensory profiles of the resulting wines are described. The field experiment implemented a leaf removal treatment in the fruit zone on the morning side and its control, in combination with the implementation of UVB exclusion sheets in each. Four microclimates were therefore established and confirmed/validated to modulate light quantity (exposure level) and quality (presence/absence of UVB).

The basic wine parameters measured in this study confirmed that all the wines were fermented to dryness without any major differences observed. The fermentations were therefore similar in terms of fermentation parameters and only slight differences were seen, most notably the slightly higher level of residual sugar, and the higher fructose: glucose ratio in the HLcontrol samples. These differences were statistically insignificant, but it could be argued that this treatment created a slightly suboptimal fermentation environment in the juice. The juice analyses did show that the HLcontrol samples had the lowest total amino acid pool that could have impacted slightly on the fermentation performance (discussed in Chapter 5). Furthermore, a microbiome study that was conducted in the same experimental vineyard in the HL and LL environments (the analysis did not include the UVB attenuation treatments) showed that there were clear differences in natural microorganism population size and composition in juices obtained from the HLcontrol and LLcontrol microclimates (Morgan, 2016). The HL microclimate displayed a decreased presence of microorganisms, similar to other studies that have also linked an increase in light exposure and UV radiation to reduced microbial loads (Daniel O. and Marois, 1992; Zoecklein et al., 1992).

6.4.2 Possible links between volatiles, glutathione and oxidation status of the HL environments

The modulation of berry microclimate also lead to perceptible differences in the wine sensorial characteristics (Figure 6.5 and 6.6). Various studies in Sauvignon Blanc have shown that leaf removal and the consequent increase in light will reduce the green characteristics of the wine and will enhance the more tropical and fruity flavours and aromas (Coetzee and du Toit 2012 and references therein). Similar results were seen in this study in both experimental seasons; however the effects of UVB attenuation revealed interesting responses within the two light exposures. Interestingly, the sensory data showed that the impacts of the exposure level (HL vs LL) were negated to a degree with attenuation of UVB as the HL-UVB and LLcontrol were closely grouped together (Figure 6.5) and shared descriptors such as “fresh pineapple and “cooked vegetable”. From our data it was clear that the UVB component of light is essential in activating the metabolism linked to the “tropical” descriptors.

These tropical characteristics associated with the HLcontrol wines were described with descriptors that are strongly linked to specific thiol compounds. The volatile thiols constitute an important group of odour impact compounds in Sauvignon Blanc and contribute to the tropical, grapefruit, passionfruit and guava aromas. The main thiols present in Sauvignon Blanc wine include 4-mercapto-4- methylpentan-2-one (4MMP), 3-mercaptohexan-1-ol (3MH) and 3-mercaptohexyl acetate (3MHA). These grape-derived volatiles were not measured in the bottle aged wines, as focus was given to fermentation derived-aroma compounds, but the thiol-related sensory descriptors in the wines are consistent with data that was presented by Suklje et al. (2014), who conducted a study in the same experimental vineyard, also demonstrating a significant decrease in thiols with UVB attenuation under HL conditions. Kobayashi et al. (2011) reported increased production of thiol precursors in the grapes exposed to higher UVB levels. The increased hexanal in HL control berries may also be related to higher thiols as it reacts with glutathione to produce the pro-precursor Glut-3MH which is catalysed to for the precursor Cys-3MH and finally the thiol 3MH. Similarly, C6 compounds, including hexanal were found to be higher in the HLcontrol grape berries. As was discussed in chapter 3, these compounds are synthesised via the LOX-HPL pathway which plays as important role in stress signalling. It was shown that UVB contributes to the release of certain C6 compounds implicating it the regulation of this pathway and therefore the metabolism of PUFA's. Taken together, the UVB induced levels of glutathione and hexanal in the HLcontrol grape juice may have led to higher levels of 3-MH, consequently contributing to the passionfruit character of the wine. The results therefore provide strong support for the notion that UVB is important in the formation of thiol-precursors, and here we show that the UVB effects are dependent on the overall exposure level of the grapes as well.

Furthermore, juices prior to fermentation were seen to be less brown in the HLcontrol juices compared to the others as is demonstrated in Chapter 5. Glutathione levels present during fermentation will be linked to those present in the juice (Kritzinger et al., 2013). Given glutathione's role as an antioxidant and the different levels measured in the four juices from the four microclimates in this study (refer to Chapter 5) the oxidation status of the juices/wines are important to consider in terms of the volatile components. The HLcontrol juice samples appeared to have more oxidation buffering capacity in comparison with the other samples and in particular the LL environments and the UVB attenuation samples. Variations in glutathione levels have been shown to contribute to different oxidation potentials in juices with impacts on fermentation and the composition of volatile compounds formed. Glutathione reduces the oxidation-related browning of juice and also preserves certain aroma impact compounds in wine (Papadopoulou and Roussis, 2008; Singleton et al., 1985; Ugliano et al., 2011).

Suklje et al. (2014) hypothesised that higher levels of glutathione and lowered levels of grape reaction product measured in the must of grape samples exposed to HL conditions may also have contributed to higher thiol production. The grape reaction product, also known as 2-(S)-glutathionyl caftaric acid, is created through the reaction between glutathione and ortho-quinones (Cilliers and Singleton, 1990;

Kritzinger et al., 2013). Another study by Suklje et al. (2016) suggested that dry yeast application to berries in the vineyard led to a preservation of aromas in wine by increasing the antioxidants, namely glutathione and certain amino acids. Glutathione is necessary for the formation of glutathione-3-mercaptohexan-1-ol (Glut-3MH), a precursor to 3MH which contributes to the passionfruit aroma in wine (Kilmartin et al., 2015), a descriptor that was consistently linked to the HLcontrol wines over multiple seasons.

Interestingly, the "hay" and "dried fruit" characters perceived in the HL-UVB wines, as well as the "dusty" character picked up in the LLcontrol wines in the second season may be further indications of altered oxidation potential of the different juice/wine matrices as these characters were found in oxidised wines (Chisholm et al., 1995; Silva Ferreira et al., 2003). It has also been shown that supplementation of juice with glutathione can increase the production of esters (Pinu et al., 2014). One of the key results from the wine analysis was the fact that the HLcontrol wines had significantly higher levels of esters and the corresponding juice was increased in glutathione.

Related to the oxidation state of the different matrices, the grapes as well as the juices were shown to be affected in their polyphenolic content. Typically a decreased accumulation under attenuated UVB levels in the HL microclimate was seen and interpreted as an acclimation strategy to mitigate UVB related stress and maintain antioxidant homeostasis (Chapter 3). Similarly, increased phenols were also noted in the corresponding juice (HLcontrol), as described in Chapter 4. The extraction of these compounds from berries has been shown to contribute to the antioxidant capacity of the wine during fermentation, also leading to the preservation of aromatic thiols (Coetzee and du Toit, 2015; Kilmartin et al., 2015; Olejar et al., 2015a), and therefore provides further support and context for the sensory profiles that infer higher thiol content in the HLcontrol wines. The absence of these descriptors in the UVB-attenuation samples confirm the important role of UVB exposure on their precursor formation in the berries and generating a matrix that can support their maintenance and stability. These results motivate for a careful analysis of the different matrices in terms of their antioxidant potential and the overall monitoring of oxidation status when considering the exposure and UVB attenuation effects in future.

6.4.3 The fermentation-derived chemical profiles confirm amino acids as important drivers in the wine profiles.

The greatest responses in the fermentation-derived volatile compounds were seen in the dry wine samples with each microclimate exhibiting different higher alcohol, fatty acid and ester profiles (Figure 6.3). A number of these responsive compounds could be related to amino acid metabolism via the Ehrlich pathway (Figure 6.4). Typically, the nonpolar branched-chain amino acids (valine, leucine and isoleucine) and phenylalanine are metabolized through this reaction and form the most important odour related products including certain higher alcohols and their associated fatty acids and esters (Hazelwood

et al., 2008; Lilly et al., 2006; Styger et al., 2011; Wang et al., 2016a). The specific compound formed will be dependent on the original amino acid present (Styger et al., 2011; Swiegers et al., 2005). Boss et al. (2015) also confirmed the importance of amino acid availability and composition in determining the volatile aroma compound production in wines. More recently, the volatile composition of wine was shown to be dynamic with many changes occurring in relation to the initial grape composition. It was concluded that the changes observed in wine volatile composition could be as a result of yeast responses to changing musts during fermentation, or a direct reflection of the differences noted in grape-derived precursors (Boss et al., 2017).

The esters were seen to be the most responsive in our analysis of fermentation-derived volatiles, particularly in the HL microclimate. (Figures 6.2 and 6.3). Pinu et al. (2014) showed that when juice was supplemented with 14 chosen amino acids, a significant increase in aroma compound production occurred, including higher alcohols and esters, while a reduction in the levels of certain fatty acids were seen. Similarly, total esters and higher alcohols were seen to be higher in the LL microclimate wines (Figure 6.2) which may be related to the higher levels of total amino acids seen in the LL microclimate settled juices compared to the HL juices (Chapter 5). Furthermore, in the HL bottle aged wines, UVB attenuation showed higher levels of these compound pools. This may also be linked to the decreased amino acids available in the HLcontrol settled juices compared to the HL-UVB juice. Additionally, Chapter 5 highlighted the differences in BCAA composition in the settled juice samples (highest in HL-UVB). These responses were related to the variations in UVB, particularly in the HL microclimate. The differential availability of the BCAAs for the different fermentations are therefore interpreted to have contributed to the differential compositions of fatty acids and esters. Moreover, valine has also been implicated in the synthesis of CoA (acetyl-Coenzyme A), an important factor in the ester formation pathway (Boss et al., 2015; Saerens et al., 2010). In the HL microclimate, valine was increased with UVB attenuation in the settled juice which could have possibly been involved in the observed increase in esters in the HL-UVB bottle wines. This increase in esters could particularly have contributed to the sweet, “banana candy” character in the wine (refer to descriptors used by the trained sensory panel).

6.4.4 The potential impact of polyunsaturated fatty acids (PUFAs) on wine aromatic characteristics.

The modulation of wine aroma compounds, including the esters, in the different wines may also be linked to the differential presence of polyunsaturated fatty acids (PUFA’s). Chapter 3 described the variations seen between treatments in polyunsaturated fatty acid levels in the grape berries. It was shown that the UVB component of light contributed to the release of C_6 compounds implicating UV in the regulation the LOX-HPL pathway and consequently the metabolism of PUFAs. Higher C_6-compound levels were noted in the HLcontrol ripe berries compared to the HL-UVB. Interestingly, in the LL environment, the LLcontrol berries had similar C_6-compound levels to the HLcontrol, but significantly

lower levels relative to the LL-UVB microclimate. This could have contributed to the "green pepper," "asparagus" and "grassy" characters in the LL-UVB microclimate wines.

The presence of fatty acids plays a significant role on yeast metabolism and their initial concentration in the berries and juice may therefore influence the production of fermentation end products. Casu et al. (2016) demonstrated the influence of the PUFA linoleic acid in grape juice on important aromatic related compounds including terpenes, ethyl esters, acetate esters, fatty acids and amino acids. Pinu et al (2014) showed that an increase in linoleic acid significantly impacted the development of varietal thiols in Sauvignon Blanc. Acetate ester formation is catalysed by the enzyme alcohol acetyltransferase (Verstrepen et al., 2003). Studies conducted on ester formation have noted that unsaturated fatty acids repress the transcription of the *ATF1* gene leading to a decrease in acetate ester production (Fujiwara et al., 1998; Mason and Dufour, 2000; Swiegers et al., 2007). This has been demonstrated in Sauvignon Blanc (Casu et al., 2016). Our data further corroborates this as significantly increased levels of higher alcohols were seen in the LL-UVB dry wine samples. This may be due to a reduction in alcohol acetyltransferase activity as a result of the higher incidence of fatty acids. Similarly, regarding ethyl esters, the presence of PUFA's in the fermentation medium has been shown to suppress their formation (Saerens et al., 2008; Sumby et al., 2010).

The lipidome of berries and juice may therefore be considered an important factor influencing the fermentation process and the formation of certain aromatic compounds. This provides further evidence that the modification of the bunch microclimate will impact wine composition and sensorial attributes. In the study of wine organoleptic properties however, it is important to note that during aging, the wine undergoes various modifications in composition through chemical alterations as constituents move closer to their points of equilibrium, leading to changes in flavour and aroma (Sumby et al., 2010). Generally, a loss of varietal and fermentation related characteristics are noted during wine aging. Similarly, the results of this study showed a significant suppression of treatment effects in the different volatile compounds as well as a decrease in many volatile compounds. In Sauvignon Blanc, this loss in varietal characteristics has been attributed to the degradation of esters through the process of chemical hydrolysis (Coetzee and du Toit, 2015; Lambropoulos and Roussis, 2007; Ramey and Ough, 1980; Sumby et al., 2010); oxidation; or by the direct interaction with o-quinones (Patrianakou and Roussis, 2013). Typically the acetate esters will degrade at a faster rate, while the ethyl esters degrade more slowly (Ramey and Ough, 1980). Certain classes of volatiles can however be protected by a reduction in hydrolysis or change in their biosynthesis through the alteration of relevant components of the juice composition (Suklje et al. 2016).

6.5 Conclusions and perspectives

Wine is an immensely complex matrix and very rarely does a single compound contribute specifically to a certain attribute alone. The final sensory profile is rather governed by the interaction between all the present compounds; enhancing, masking or changing aromatic and flavour characteristics (Chambers and Koppel, 2013; Ferreira et al., 2016; Styger et al., 2011). Although hugely simplified, it is accepted that in general terms the presence and concertation of these influential compounds are dependent on the initial grape composition, yeast fermentation, certain wine making practices and aging. In such a "pipeline" all of the individual steps in the grape production and wine-making process can be influenced by abiotic and biotic factors, leading to a highly complex system to study. Increasingly, knowledge is generated that follows the influence of environmental factors from the grapes to the wines in an attempt to not only explain, but also predict and link certain impacting factors and their effects on the subsequent matrices and the final products (Boss et al., 2017; Calonnec et al., 2004; Niimi et al., 2017; Suklje et al., 2016). Our study made significant progress towards this goal for Sauvignon Blanc. We conclude that the results presented here on four Sauvignon Blanc wines that originate in four microclimates where light exposure and UVB attenuation was studied in combination, provide some novel insights into the grape-to-juice-to wine transitions of important metabolites. Some of the affected metabolites clearly originate in the grapes in reaction to the microclimatic factors engineered in this study and also appear as drivers of separation in the wines, whereas others feed into the yeast metabolism and become influential sensory factors by contributing to fermentation-derived compounds. Other studies in this specific experimental vineyard have already highlighted the effects of UVB on significant aroma-related compounds in Sauvignon Blanc, such as the thiols and methoxypyrazines (Suklje et al., 2014) and the combination of those results and our profiling of the fermentation-derived compounds provide an excellent chemical signature of the different wines which complements the sensory profiles obtained very well.

Overall, the results show the significant influence of berry microclimate on modulating grape composition, leading to altered juice and wine matrices and ultimately perceivable differences in the wines. By evaluating some of the compositions over different processing steps, the dynamic nature of these compounds becomes apparent, highlighting several risks that should be taken into account. For example, it was clear from the results that the oxidation status of the matrices were probably affected by some of the treatments. Future work should take this into account, also measuring the levels of other important influential factors, such as oxygen and quinones to contextualise the results from this perspective as well.

6.6 References

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Supplementary data of Chapter 6

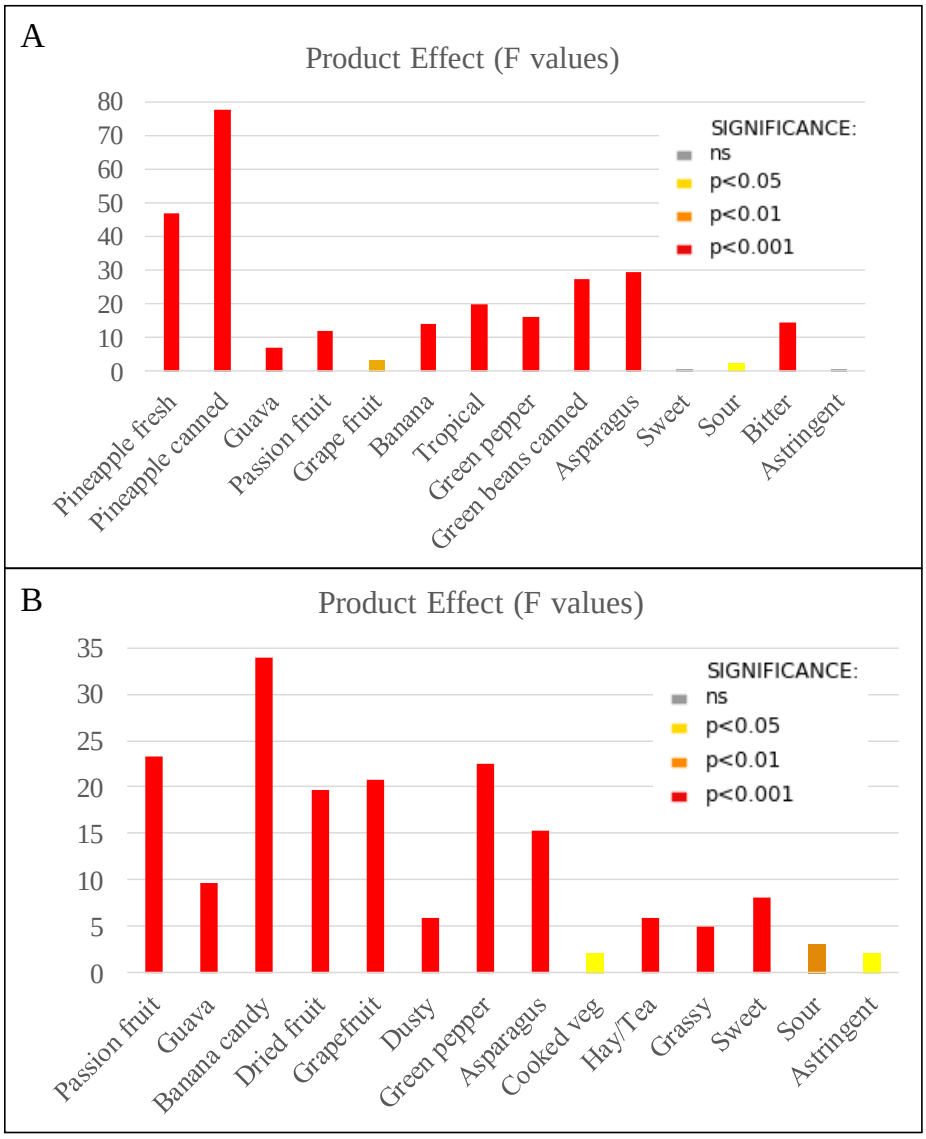
Supplementary table 6.1. A table listing the measured contents of all the fermentation derived volatile compounds ± SD for both experimental seasons. The p-values between the HL control/ HL-UVB and LL control)/LL-UVB contrasts are calculated and listed for each compound for each wine. Values highlighted in red indicate significant difference based on pairwise t-tests ($p \leq 0.05$).

Fermentation derived volatile compounds (mg/L)	Dry wine after the end of alcoholic fermentation (2014/2015)					
	HLcontrol	HL-UVB	p-value	LLcontrol	LL-UVB	p-value
Ethyl Acetate	74.1±1	68±3.6	0.00	76.1±4	75.9±1.5	0.84
Methanol	46±1.2	37.4±2.9	0.00	28.7±1.2	40.5±1.4	0.00
Ethyl Butyrate	0.9±0.1	0.9±0	0.00	0.8±0.1	0.7±0	0.00
Propanol	59.5±1.6	56.7±4.1	0.00	79.2±2.4	79.2±2.3	0.99
Isobutanol	21.2±0.5	21.5±1.2	0.18	22.9±0.6	23.7±1	0.00
Isoamyl Acetate	8.9±0.2	7.2±1.2	0.00	8.4±1.5	6.7±0.7	0.00
Butanol	1±0	1.1±0	0.08	1.1±0.1	1.2±0	0.00
Isoamyl alcohol	180.7±3.7	195.3±12.6	0.00	185.7±6.7	188.4±7	0.16
Ethyl Hexanoate	2.6±0.1	2.2±0.3	0.00	1.9±0.5	1.5±0.1	0.00
Pentanol	0.2±0	0.2±0	0.00	0.2±0	0.2±0	0.00
Hexyl Acetate	0.5±0	0.5±0.1	0.02	0.5±0.1	0.5±0	0.01
3-methyl-1-pentanol	0.4±0	0.4±0	0.00	0.4±0	0.4±0	0.00
Ethyl Lactate	4.9±0	5.2±0.3	0.00	5.3±0.2	6.5±0.1	0.00
Hexanol	0.6±0	0.8±0.1	0.00	0.8±0	0.9±0.1	0.00
3-ethoxy-1-propanol	7±0.6	6.9±1	0.92	9±0.8	8.9±0.7	0.63
Ethyl Caprylate	2.8±0.2	2±0.4	0.00	1.3±1	1±0.1	0.07
Acetic Acid	231.6±2.8	276±20.9	0.00	218.8±2.4	274.6±16	0.00
Propionic Acid	3.1±0.1	3.1±0.4	0.44	2.5±0.2	2.1±0.1	0.00
Isobutyric acid	1.1±0	1.2±0.1	0.00	1.1±0	1.2±0	0.00
Ethyl Caprate	0.3±0	0.3±0	0.00	0.3±0	0.3±0	0.16
Butyric Acid	10.1±1.7	6.4±0.7	0.00	5.9±2.8	3.5±0.3	0.00
Iso-Valeric Acid	1.3±0	1.4±0.1	0.00	1.3±0	1.3±0	0.00
Valeric Acid	1.2±0	1.2±0	0.06	1.2±0	1.2±0	0.36
ethyl phenylacetate	1±0	1.2±0.1	0.00	0.8±0	0.7±0	0.00
2-Phenylethyl Acetate	1.6±0.1	1.3±0.1	0.00	1.3±0.2	0.9±0.1	0.00
Hexanoic Acid	6.7±0.3	6.6±0.2	0.17	6.9±0.5	6.8±0.6	0.47
2-Phenyl Ethanol	23.6±0.6	26.2±3.4	0.00	18.4±0.9	17.9±1	0.08
Octanoic Acid	11±0.5	10.5±0.4	0.00	10.7±0.9	10.3±1.1	0.17
Decanoic Acid	4.1±0.2	3.5±0.3	0.00	3.4±0.5	2.8±0.3	0.00
Total esters	97.7±1.2	88.6±4.9	0.00	96.8±6.1	94.6±2.2	0.09
Total fatty acids	38.7±2.6	33.9±1.9	0.00	32.9±4.8	29.2±2.2	0.00
Total alcohols	340.2±5	346.5±14.8	0.04	346.4±9.4	361.3±7.9	0.00
Ethyl esters	11.5±0.4	10.5±0.3	0.00	9.6±1.4	9.9±0.2	0.34
Acetate esters	12±0.3	10.1±1.3	0.00	11±1.8	8.8±0.8	0.00

Fermentation derived volatile compounds (mg/L)	Young wine after cold stabilisation (2014/2015)					
	HLcontrol	HL-UVB	p-value	LLcontrol	LL-UVB	p-value
Ethyl Acetate	75.1±1.1	72.9±5.3	0.1	78.7±4.1	75.7±3.5	0.01
Methanol	47.1±1.4	41.3±1.9	0.0	32.9±1.3	41.2±2.3	0.00
Ethyl Butyrate	0.9±0	0.9±0.1	0.0	0.8±0.1	0.8±0	0.00
Propanol	58.7±1.3	59.6±6	0.4	80.5±5.5	76.7±4.2	0.01
Isobutanol	21.2±0.5	22.9±1.5	0.0	23.5±1.1	24±1.6	0.14
Isoamyl Acetate	8.3±0.5	7.2±1	0.0	8.6±1.4	8.4±0.9	0.43
Butanol	1±0	1.1±0	0.0	1.2±0.1	1.2±0.1	0.00
Isoamyl alcohol	181.9±6.4	201.3±18.8	0.0	190.1±6.6	200.8±12	0.00
Ethyl Hexanoate	2.1±0.1	1.9±0.1	0.0	2±0.4	1.8±0.2	0.00
Pentanol	0.2±0	0.2±0	0.0	0.2±0	0.3±0	0.00
Hexyl Acetate	0.4±0	0.4±0	0.2	0.5±0.1	0.6±0	0.78
3-methyl-1-pentanol	0.4±0	0.4±0	0.0	0.4±0	0.4±0	0.52
Ethyl Lactate	5.3±0.1	5.6±0.1	0.0	5.7±0.2	6.1±0.2	0.00
Hexanol	0.6±0	0.8±0.1	0.0	0.8±0	0.9±0.1	0.00
3-ethoxy-1-propanol	7±0.4	7.3±1.1	0.2	9.8±1.4	9.7±0.9	0.83
Ethyl Caprylate	1.2±0.2	1±0.1	0.0	1.3±0.5	0.9±0.2	0.00
Acetic Acid	231.9±5.9	291.3±25.6	0.0	233.6±20.1	291.4±19.1	0.00
Propionic Acid	2.1±0.1	2.2±0.1	0.0	2.2±0.2	2.2±0.1	0.17
Isobutyric acid	1.2±0	1.2±0.1	0.0	1.1±0	1.2±0.1	0.00
Ethyl Caprate	0.3±0	0.3±0	0.0	0.3±0	0.3±0	0.02
Butyric Acid	4.4±0.5	4±0.2	0.0	4.4±1.1	3.5±0.4	0.00
Iso-Valeric Acid	1.4±0	1.4±0.1	0.0	1.3±0	1.4±0.1	0.00
Valeric Acid	1.2±0	1.2±0	0.0	1.3±0.1	1.3±0	0.06
ethyl phenylacetate	1±0	1.2±0.1	0.0	0.8±0.1	0.8±0	0.23
2-Phenylethyl Acetate	1.2±0	1.1±0	0.0	1.1±0.1	0.9±0.1	0.00
Hexanoic Acid	6.8±0.5	6.6±0.5	0.1	6.8±0.5	7.2±0.4	0.00
2-Phenyl Ethanol	23.7±1.1	25.8±3.9	0.0	18.6±1	18.8±1.5	0.64
Octanoic Acid	10.7±0.9	10.1±0.8	0.0	10.2±0.9	10.7±0.8	0.05
Decanoic Acid	3.1±0.3	2.8±0.2	0.0	3±0.4	2.7±0.5	0.06
Total esters	95.7±1.8	92.5±6.3	0.0	99.9±6.4	96.2±4.6	0.02
Total fatty acids	30.8±1.8	29.5±1.9	0.0	30.3±2.4	30.2±2.4	0.95
Total alcohols	341.8±8.5	360.7±22.7	0.0	357.9±11.9	374.1±19.9	0.00
Ethyl esters	9.8±0.3	9.7±0.4	0.7	10.1±0.8	9.9±0.6	0.27
Acetate esters	10.9±0.5	9.8±1	0.0	11.1±1.6	10.6±0.9	0.23
Fermentation derived volatile compounds (mg/L)	**Bottle aged wine after 8 months (2014/2015)**					
	HLcontrol	**HL-UVB**	**p-value**	**LLcontrol**	**LL-UVB**	**p-value**
Ethyl Acetate	61±1.4	65.5±1.9	0.08	61.8±1.3	66.9±0	0.08
Methanol	52±1.7	45.7±1.4	0.02	47.2±3.5	45.5±0	0.72
Ethyl Butyrate	0.8±0	0.8±0	0.77	0.7±0	0.7±0	0.41
Propanol	56.9±3.4	63.9±11.6	0.48	66.6±12.6	72.8±0	0.71
Isobutanol	19.5±0.7	20.3±1.4	0.48	21.4±1.4	20.8±0	0.74

Isoamyl Acetate	3.9±0.2	4±0.2	0.51	3.9±0.2	4.1±0	0.50
Butanol	1.1±0	1.1±0.1	0.20	1.1±0	1.2±0	0.16
Isoamyl alcohol	169.2±5	177.6±7.6	0.30	181.6±16.3	174.4±0	0.74
Ethyl Hexanoate	1.8±0.1	1.7±0.1	0.16	1.7±0.1	1.6±0	0.45
Pentanol	0.3±0	0.3±0	0.21	0.3±0	0.3±0	0.23
Hexyl Acetate	0.2±0	0.2±0	0.17	0.3±0	0.3±0	0.48
3-methyl-1-pentanol	0.3±0	0.3±0	1.00	0.3±0	0.3±0	0.60
Ethyl Lactate	17.5±0.7	18.2±2	0.66	19.9±1.3	19.9±0	0.99
Hexanol	1±0	1.2±0	0.04	1.3±0.1	1.3±0	0.42
3-ethoxy-1-propanol	7.5±0.9	8±1.3	0.69	8.9±0.2	9±0	0.66
Ethyl Caprylate	1.4±0.2	1.3±0.3	0.74	1.4±0.1	1.3±0	0.38
Acetic Acid	236.1±12.2	263.3±23.4	0.24	242.2±2.9	266.3±0	0.02
Propionic Acid	2.6±0.1	2.5±0.2	0.58	2.4±0.2	2.3±0	0.69
Isobutyric acid	1.1±0	1.1±0	0.59	1.1±0.1	1±0	0.70
Ethyl Caprate	0.5±0.1	0.5±0.1	0.92	0.7±0.1	0.5±0	0.21
Butyric Acid	2.2±0.1	2.2±0.1	0.99	2.2±0.1	2±0	0.35
Iso-Valeric Acid	1.2±0	1.2±0	0.83	1.2±0.2	1.1±0	0.78
Valeric Acid	0.4±0	0.4±0	0.90	0.4±0	0.4±0	0.57
ethyl phenylacetate	0.2±0	0.1±0	0.85	0.1±0	0.1±0	0.07
2-Phenylethyl Acetate	0.6±0	0.6±0	0.24	0.6±0	0.6±0	0.53
Hexanoic Acid	7.2±0.3	7.1±0.3	0.85	7±0.3	7.1±0	0.94
2-Phenyl Ethanol	23.5±0.7	21.6±2.6	0.42	18.4±1.7	16.7±0	0.47
Octanoic Acid	11±0.5	10.7±0.4	0.61	10.5±0.6	10.7±0	0.80
Decanoic Acid	3.3±0.2	3.2±0.2	0.66	3.4±0.2	3.3±0	0.69
Total esters	90±1.7	95±2.4	0.11	92.9±1.5	97.7±0	0.11
Total fatty acids	29.1±0.9	28.7±-0.1	0.24	28.2±0.5	28.1±0	0.84
Total alcohols	331.3±11.5	340.1±19.1	0.62	347.1±13.1	342.4±0	0.79
Ethyl esters	24±0.4	24.5±1.6	0.74	26.2±1.2	25.7±0	0.75
Acetate esters	4.9±0.2	5±0.2	0.45	4.8±0.2	5.1±0	0.48
	Bottle aged wine after 8 months (2013/2014)					
	HLcontrol	**HL-UVB**	**p-value**	**LLcontrol**	**LL-UVB**	**p-value**
Ethyl Acetate	60±0.9	59±0.6	0.36	59.4±1	55.4±1	0.00
Methanol	53.6±1.5	51.4±1	0.21	45.9±1.2	44.3±0.9	0.03
Ethyl Butyrate	0.8±0	0.8±0	0.68	0.7±0	0.7±0	0.02
Propanol	80.9±1.9	78.8±0.7	0.36	87±1.7	76.4±1.3	0.00
Isobutanol	24.1±0.3	23.8±0.2	0.44	23.5±0.3	20.9±0.4	0.00
Isoamyl Acetate	4.3±0.1	4.4±0	0.11	3.7±0	4±0.1	0.00
Butanol	1.2±0	1.2±0	0.29	1.1±0	1.2±0	0.06
Isoamyl alcohol	197±0.7	197.1±0.5	0.92	186.4±0.3	175.1±2.9	0.00
Ethyl Hexanoate	1.8±0	1.9±0	0.08	1.7±0	1.6±0	0.00
Pentanol	0.2±0	0.2±0	0.57	0.2±0	0.2±0	0.00
Hexyl Acetate	0.4±0	0.4±0	0.11	0.4±0	0.4±0	0.00
3-methyl-1-pentanol	0.4±0	0.4±0	0.58	0.4±0	0.4±0	0.00
Ethyl Lactate	25.6±0.3	25.2±0.2	0.38	24.6±0.1	24.9±1.6	0.73

Hexanol	1.4±0	1.4±0	0.04	1.5±0	1.3±0	0.00
3-ethoxy-1-propanol	7.3±0.1	7.1±0.1	0.42	7.7±0	7.2±0.2	0.00
Ethyl Caprylate	2.3±0	2.4±0	0.09	2.2±0	2.1±0	0.04
Acetic Acid	213.5±1.8	210.8±0.7	0.19	232.4±1.5	195.2±4	0.00
Propionic Acid	2.6±0	2.6±0	0.35	2.7±0	2.2±0.1	0.00
Isobutyric acid	1.2±0	1.2±0	0.60	1.3±0	1.2±0	0.00
Ethyl Caprate	5.3±0.1	5.4±0.1	0.55	5.7±0.2	5.1±0.1	0.00
Butyric Acid	0.3±0	0.3±0	0.93	0.3±0	0.2±0	0.02
Iso-Valeric Acid	1.5±0	1.5±0	0.83	1.5±0	1.4±0	0.00
Diethyl Succinate	1.7±0	1.7±0	0.02	1.5±0	1.4±0	0.00
Valeric Acid	1±0	1±0	0.77	1±0	1±0	0.00
ethyl phenylacetate	0.3±0	0.3±0	0.63	0.3±0	0.3±0	0.00
2-Phenylethyl Acetate	0.8±0	0.8±0	0.34	0.8±0	0.7±0	0.01
Hexanoic Acid	7.1±0.1	7.3±0	0.05	6.7±0.1	6.6±0.1	0.06
2-Phenyl Ethanol	21±0.2	21.2±0.2	0.23	20.2±0.1	18±0.4	0.00
Octanoic Acid	11±0.2	11.4±0.1	0.07	10.5±0.1	10.5±0.2	0.90
Decanoic Acid	3.5±0.1	3.6±0	0.26	3.6±0.1	3.5±0	0.00
Total esters	103.4±1	102.4±0.6	0.45	101.1±0.9	96.8±1.9	0.00
Total fatty acids	28.3±0.3	28.8±0.2	0.12	27.5±0.3	26.4±0.4	0.00
Total alcohols	387.2±4.3	382.7±1.7	0.40	374±3.2	345±5.7	0.00
Ethyl esters	37.5±0.2	37.4±0.2	0.69	36.5±0.2	35.9±1.7	0.44
Acetate esters	5.9±0.1	6±0	0.10	5.3±0	5.5±0.1	0.00



Supplementary figure 6.1 Product effect in the two-way ANOVA model expressed as F-values for the 2013/2014 season (A) and the 2014/2015 season (B). Significant attributes are indicated according to their associated p-values.

Chapter 7

General conclusions and perspectives

In the study of UVB effects in plant systems it has become evident that rarely do UVB induced damage phenotypes come to the fore. Instead, under field conditions, which represent a more realistic UVB exposure situation, plants are able to acclimate through various mechanisms including morphological changes (Tilbrook et al., 2013), the modulation of metabolites (Morales et al., 2013; Shen et al., 2017) and the transcript accumulation of genes related to UV acclimation and tolerance (Favory et al., 2009; Morales et al., 2013). Many of these processes have furthermore been found to be regulated through UVB signaling via the UVR8 photoreceptor. (Singh et al., 2017b; Sinha et al., 2014; Tilbrook et al., 2013).

Grapevine related UVB studies were limited before the commencement of this PhD in 2013, particularly those performed under natural field settings. Since then, several important trials have been conducted looking at the molecular and metabolic responses of grapevine under ecologically relevant UVB doses, not in terms of damage, but rather as a way to better understand the underlying mechanisms involved in stress mitigation. A number of approaches have been utilized to investigate UVB impacts in field trials, including the use of UVB screens installed over the bunch zone, encasement of the bunches in UV attenuating bags and the complete covering of the grapevine in plastic films with different UV transmittance properties (De Oliveira et al., 2015; Gregan et al., 2012; Koyama et al., 2012b; Liu et al., 2015b). Evidence from these studies has revealed that grapevine is in fact well adapted to UVB exposure and typically shows acclimation responses that include morphological (Del-Castillo-Alonso et al., 2016; Doupis et al., 2016), metabolic (Del-Castillo-Alonso et al., 2016; Reshef et al., 2018; Song et al., 2015) and transcriptomic (Liu et al., 2015b) changes, all of which contribute to the phenotypic plasticity of grapevine to the UVB stress signal.

Despite these significant advancements, which coincided with the objectives of this study, the assessment of UVB impacts in white cultivar grape berries remained restricted. The results obtained from this study, which were performed as a grape to wine analysis using Sauvignon Blanc grape berries produced under four distinct microclimates, yielded novel insights and makes a contribution to our current understanding of UVB impacts on the berry and wine matrices.

The benefit of this PhD study lay in the experimental setup. A previous trial in a vineyard validated a method for the modulation of light exposure (Young et al., 2016), thus providing a basis from which to conduct UVB manipulations in the bunch zone. Furthermore, the utilisation of this previously highly characterised vineyard, with numerous climatic variables being measured over several seasons, facilitated the assessment of treatment impacts. Field studies are notoriously complicated due to the compounding effects of the multitude of variables inherent to a natural environment. This consequently

makes linking responses to specific variables difficult and lab trials are sometimes favored due the control which can be exercised in terms of variables. In answer to this problem, a field-omics approach was introduced in Alexandersson et al. (2014) which involved extensive characterisation of the vineyard as a way to account for the many environmental variables. This in conjunction with further climatic measurements including temperature, light exposure and UVB radiation allowed for the confirmation of a UVB exclusion microclimate in the bunch zone under both high light and low light conditions. This in turn facilitated the linking of certain metabolic responses to specific light quantity and quality conditions.

The main outcomes of the experimental work are contextualised in the sections below.

7.1 The responses elicited by the different UVB exposures under High light and Low light microclimates.

The modulation of the berry microclimate in terms of light quantity and quality significantly impacted various aspects of berry metabolism, as well as juice and wine properties. Figure 7.1 proposes an overview model of the respective UVB radiation elicited impacts under variable light exposure in the different matrices, highlighting the most important metabolic responses. The field-omics approach employed in this study demonstrated considerable plasticity in grapevine responses to UVB attenuation, which were modulated by the level of light exposure and influenced by the developmental stage of the berries. The alterations in berry microclimate during development clearly influenced berry composition, thereby forming four individual berry matrices at maturity. The juices yielded from these berries were distinct in their composition in each microclimate and displayed variable compositional changes during juice processing prior to fermentation. This in turn influenced the final wine composition, resulting in four wines unique to each microclimate (Figure 7.1).

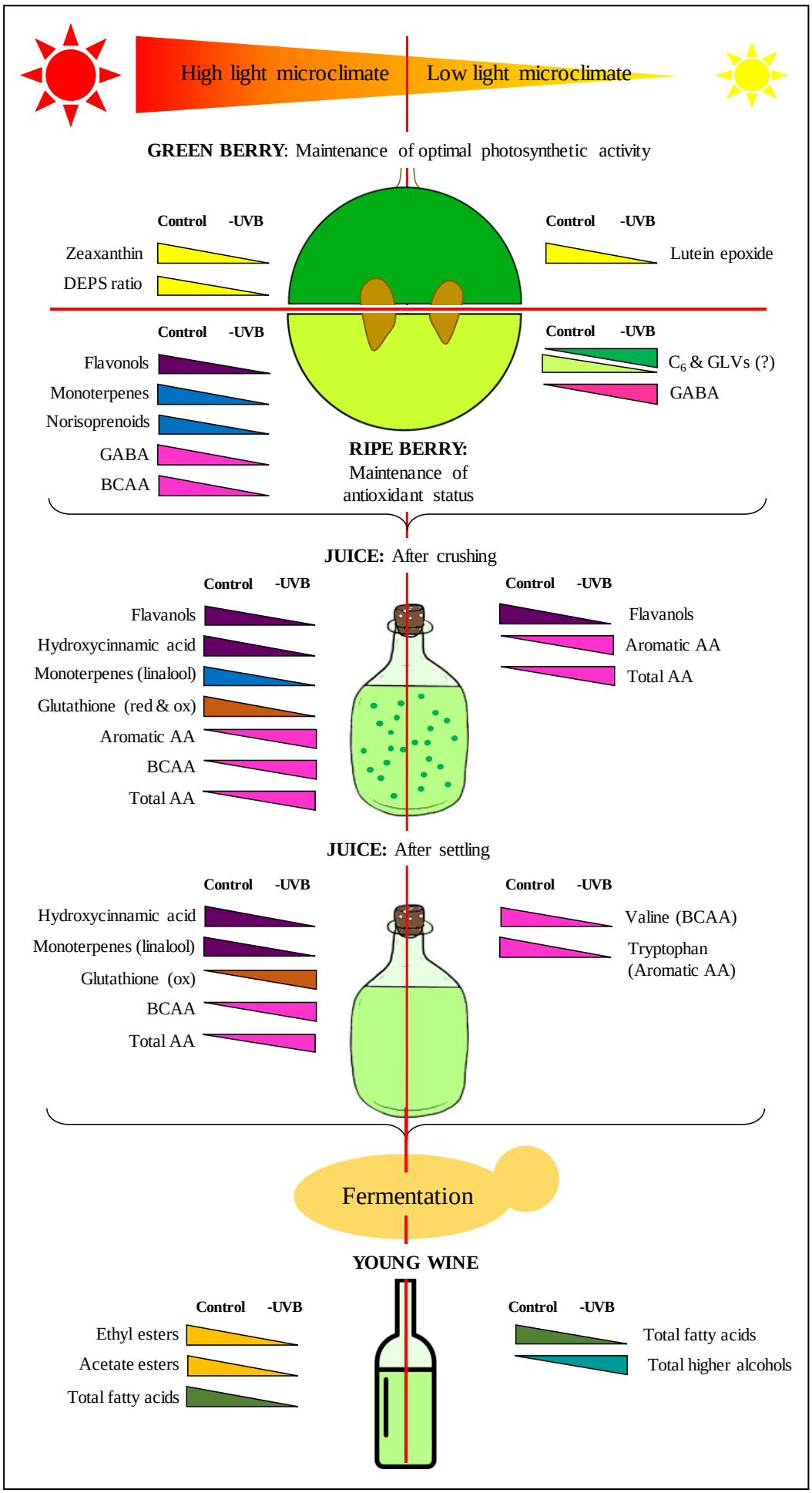


Figure 7.1. A comprehensive model summarizing the main results of the study from the green berry to the young wine. The light environments (HL and LL) are indicated with the red and yellow sun symbols respectively and the attenuation of UVB is represented for each light microclimates with the coloured triangles. These triangles indicate the main compounds which reacted to UVB attenuation in each case and show either an increase or decrease corresponding to the tapering of the triangles.

7.1.1 Grapevine berries acclimated to UVB through the modulation of certain metabolites which were dependent on developmental stage and light exposure.

The results of this study demonstrated that specific metabolites responded to the modulation of UVB radiation in a metabolically plastic manner and these responses were dependent on light exposure and developmental stage. In the green developmental stage, when berries were still photosynthetically active, specific photoprotective carotenoids, namely the xanthophylls, reacted to the differences in UVB exposure. These xanthophylls are related to the violaxanthin and lutein epoxide cycles, both of which were demonstrated to be functional in the green berries under both the HL and LL microclimates. The lutein epoxide and violaxanthin cycles have been demonstrated to optimise the light harvesting process as well as facilitate energy dissipation, however, the work done on the responses induced by UVB light in the xanthophyll cycles is minimal. Here we showed that UVB radiation could be implicated in the formation of zeaxanthin under HL conditions, the directional change in the xanthophyll cycle associated with photoprotection through energy dissipation. Furthermore, under LL conditions, the variation in UVB was implicated in the metabolism of lutein epoxide, a molecule which serves a photoprotective role under conditions of sudden and localised light exposure under shaded conditions. These results demonstrated the ability of the grape berry to shift its metabolism as a way to mitigate potential UVB damage and acclimate to the environment. The successful acclimation of green grape berries to UVB exposure was evidenced by the insignificant effect of UVB exposure on the chlorophylls and main carotenoids under both the HL and LL microclimates. These results indicated that photosynthetic processes were probably maintained in the green berries implying that the perceived stress was mitigated through certain mechanisms such as this non-photochemical quenching via the xanthophyll cycles. This point could be further validated through photosynthetic measurements of the green grape berries, however certain difficulties lie in the non-destructive determination of photosynthesis in developing fruit. Despite this, our results extended the current understanding of UVB impacts in grapevine fruits by showing that some of the specific carotenoids involved in photoprotection were responsive to levels of solar radiation (exposure), but that the UVB component in this light signal was required for the typical photoinhibition responses linked to the violaxanthin and lutein epoxide cycles. This provided novel insights into the underlying mechanisms employed by green developing grape berries to acclimate to UVB.

Ripe berries also responded metabolically to the variations in UVB under HL and LL microclimates, resulting mostly in the formation of compounds which have direct antioxidant and/or “sunscreening” abilities. The most typical UVB induced response was the accumulation of polyphenolics, most notably with higher light exposure. The phenolics have received the most attention in UVB related studies and these results served to further corroborate the typical responses seen in grape berries as well as further our insights into white cultivar responses. Furthermore, the variation in UVB induced several modulations in berry volatile compound composition. A noted increase in monoterpenes and norisoprenoids with ambient UVB assisted in the maintenance of the berry antioxidant status. The

antioxidant capacity of isoprenes has been validated in plants (Calogirou et al., 1999; Loreto et al., 2001, 2004; Velikova et al., 2004) and it is possible that this is one of their biological functions in older (sink) tissues such as ripe berries. Similarly, the norisoprenoids may serve as sensing and signaling compounds when plants are subjected to stress as a way to mitigate potential damage by activating oxidative stress defense mechanisms (Ramel et al., 2012). These ripe berries also displayed interesting amino acid responses, which were modulated depending on the level of light exposure. Foremost of these observed responses were the changes in the branched chain amino acids and GABA; amino acids which may be implicated in the mitigation of stress through their roles as metabolites or signaling compounds. Responses were most evident in the HL microclimate which showed lowered levels of GABA and BCAAs with UVB attenuation.

Taken together, it can be concluded that core processes such as photosynthesis were maintained by differentially modulating metabolites under the four treatments in the green berry, while metabolic responses in the ripe berry were employed to maintain antioxidant homeostasis and aid in stress management. It is clear from the results that grape berries were able to acclimate to UVB radiation by employing various mechanisms and metabolites. The study therefore showed that under field conditions, UVB does not induce damage characteristics in white Sauvignon Blanc berries, but is rather utilized by the plant to induce metabolic changes which allow it to cope with perceived stress, demonstrating extreme plasticity in its responses.

The regulation and control of possible pathways involved were however not explained in this study. Transcriptional investigation of the berry samples would provide invaluable insights into the regulatory mechanisms involved in berry acclimation through metabolic modulation, further explicating the process of grapevine phenotypic plasticity. Furthermore, the measurement of stress biomarkers such as the presence ROS would aid in confirming the level of stress experienced in the berry, providing further insights into UVB induced responses. Of further interest for future work would be the investigation of berry skin and pulp separately considering the natural compartmentalization of metabolites to particular berry tissues.

7.1.2 The variations in UVB elicited compositional changes to the juice matrix and affected the resulting wines.

The modulation of berry microclimate also led to changes in the juice composition at three processing stages. This novel characterization of juice during processing in relation to the variation in light quantity and quality revealed interesting results concerning the inherent nature of the berry juice. Juice characterization following harvest showed a non-linearity between the berry and juice composition following crushing and these were unique to specific microclimates. There was a clear difference between berry compositions compared to the compounds extracted into the juice. Furthermore, juice characteristics were not maintained during processing and displayed a significant variability in

composition between the crushed and the settled juice. This was most notable in the amino acids. Amino acid composition would considerably affect the fermentation as they are utilized by the yeast which consequently form an array of aromatic volatile compounds. Furthermore, the manipulation of light and UVB exposure was shown to modulate metabolites mostly located in the skin tissue of the grape berry including the monoterpenes, phenolics and amino acids. These compounds contribute to the varietal characteristics of the wines and the skin of the grape berry thus potentially represents an untapped pool of aromatic potential. Further experimentation to determine the effects of different extraction methods such as extended skin contact or the usage of specific enzymes could potentially benefit wine attributes.

To our knowledge, this is the first study demonstrating the dynamic nature of juice matrices, implying the presence of chemical or biological process which actively influence certain compounds during processing. The accepted assumption that the juice composition is stable seems to be somewhat inaccurate and knowledge of the processes involved in altering juice composition will benefit wine making decisions.

Secondly, it was interesting to note that although juices demonstrated a certain dynamic nature, four distinct compositions were maintained throughout processing, highlighting the significant effect of microclimate on juice characteristics. The metabolic changes noted in the ripe berry due to variations in light quantity and quality influenced the juice composition and therefore the fermentation. This in turn significantly influenced the wine composition. Several of the measured metabolites originated in the grapes and were modulated by the variation in light exposure and UVB radiation. Other metabolites which were influenced by the berry microclimate were utilized by the yeast and thereby contributed to the fermentation derived volatile compounds. These compositional changes did not exclusively impact fermentation, but the differences in microclimate furthermore altered the juice environment, which drastically impacted on the outcome of certain compounds. The juice oxidation status was significantly influenced by UVB through the modulation of glutathione and phenolic composition. This may have in turn affected the formation and protection of important aromatic compounds. Furthermore, the berry and juice lipidome were postulated to have influenced fermentation and therefore the wine composition. These results highlighted the influence of UVB on wine compositional properties and also provided novel insights into the grape-juice-wine transitions of certain metabolites.

7.1.3 Wine sensorial attributes were impacted by berry microclimate

The sensorial analysis of the final bottled wines following aging revealed perceptible differences associated with the four different treatments/controls. This further substantiates the fact that bunch microclimate modification will impact wine sensorial attributes. Here we highlighted the influence of UVB particularly on the wine characteristics, linking them to compositional changes in the berries, juice and wine. Certain fermentation-derived volatile compounds may have contributed to the aroma profiles of the bottle aged wines, however, a general loss in fermentation related attributes occurred during wine

aging as has been demonstrated before in white wines (Coetzee and du Toit, 2015; Lambropoulos and Roussis, 2007; Patrianakou and Roussis, 2013). The data also revealed a strong association with tropical attributes to ambient UVB levels under HL conditions, implying that the UVB component of light is necessary for the formation of compounds linked to these aromas. Furthermore, modulation to berry microclimate may have influenced the oxidation potential of the different juice and wine matrices, resulting in the occurrence of specific aromatic descriptors. The potential impact of polyunsaturated fatty acids on wine aroma characteristics was also considered. These compounds were modulated by the variation in UVB radiation under HL and LL microclimates and may have contributed to the "green" characters associated with certain wines. Other studies conducted in the same vineyard revealed the impacts of UVB on other important Sauvignon Blanc aromatic compounds including the thiols and methoxypyrazines (Suklje et al., 2014). The combination of these results and our profiling of the fermentation-derived compounds provides a chemical signature which links to the sensorial profiles of the four wines. This is represented by Figure 7.2.

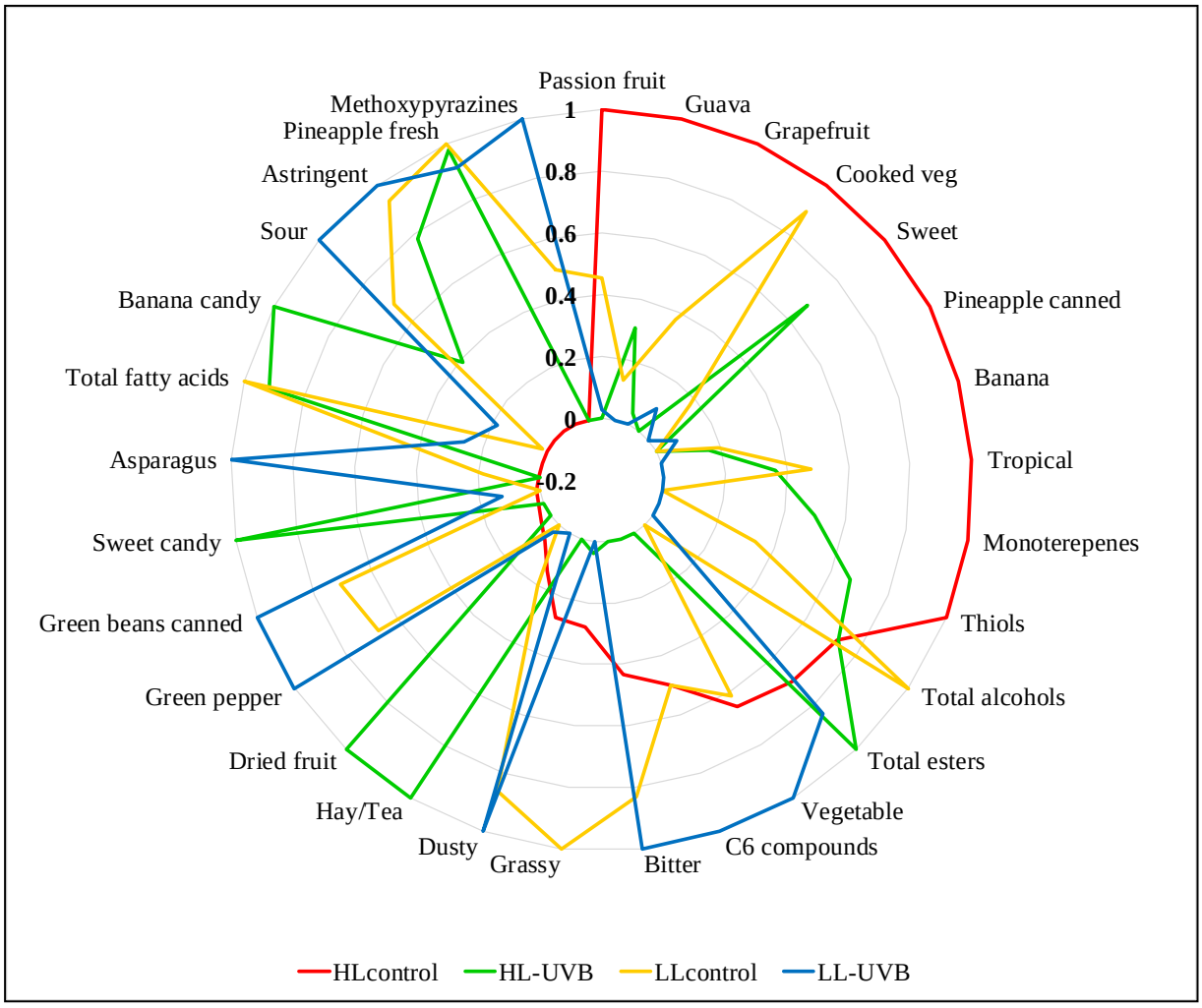


Figure 7.2. A representation of the main aromatic compound groups measured in the bottled wines as well as the sensorial descriptors associated with each wine. The graph was constructed from data attained in this study as well as the results presented in the study by Suklje et al. (2014). The data was normalised in order to better visualise the impacts of UVB in each wine.

The intricate relationships between the chemical compositions of the berry, juice and wines, combined with the effects of the microorganisms present during fermentation and storage and the ongoing chemical and biological changes occurring throughout the winemaking process contribute to the final organoleptic properties of the wine. Despite these complexities, the end result revealed four unique wines, confirming the influence of UVB radiation. Taken together, it can be said that variations in berry microclimate, such as the modulation of light and UVB, will influence the final wine, however, the links between the berry and the wine are not direct. Direct comparisons between the berry and the resulting wine are perhaps somewhat oversimplified as the relationship is far more complex. This research has revealed many novel intricacies involved in the formation of aromatic profiles and has laid the groundwork for future studies to delve into them in more detail. The confirmation of certain grape-derived aroma compounds in the bottle aged wines, as well as the monitoring of oxidation state of the wines would be useful to provide a more complete chemical/sensory integration of the wines.

7.2 General conclusions and future perspectives

This study has generated a number of novel results in the different matrices and has provided new insights into the metabolic transitions from the grape to the juice to the wine. The findings presented in this thesis have extended our knowledge of UVB acclimation responses in a white grape cultivar, highlighting the significant role of the xanthophyll cycles in the green berries, as well as the volatiles, phenols and amino acids in the ripe berry. It has furthermore confirmed the influence of microclimate on wine properties but has provided novel insights into the variations of characteristics at different points in the winemaking process, specifically in the juice.

One of the strengths of this study lay in the use of a characterised vineyard and the experimental setup. Utilising a field-omics approach, a more accurate representation of grape berry responses to UVB was generated. Furthermore, the characterisation of berries from the green developmental stage through to harvest provided a unique opportunity to follow berry responses throughout growth and ripening, thereby revealing important metabolic mechanisms employed by white grape cultivars to acclimate to UVB. The data generated from the berry samples would greatly benefit from another data layer in the form of a transcriptomic and/or proteomic analysis. Results generated from these types of analyses would give a clearer idea of the underlying metabolic pathways and genetic regulation and changes involved in berry acclimation to UVB, thereby reinforcing the presented hypotheses and conclusions.

The berry metabolites were followed through to the juice, which were for the first time characterised in terms of composition though the different processing stages. This revealed the complex nature of berry juices, opening up an array of potential future studies. The transition from the berry to the fermentation is not direct and it can be said that a number of chemical and/or biological processes are taking place in the juice. Furthermore, the modulation of the berry microclimate altered the juice environment, which

also influenced the fates of certain important compounds. Monitoring of these changes and processes during juice preparation in relation to variations in light quality would be very beneficial in furthering our understanding of UVB impacts on juice characteristics.

Ultimately, these results provide a good synopsis and novel insights into the impacts of UVB under HL and LL exposure over the entire winemaking process, starting from the green berry and ending with the wine sensorial properties. Certain compounds are translated directly from the berry to the juice and wine, including compounds such as the monoterpenes, norisoprenoids and phenolic compounds. Other compounds such as the amino acids undergo various changes through the wine making procedure and it would be important to follow these responses through the different matrices to better understand the underlying mechanisms involved in these dynamic responses. A number of additional measurements and assessments can be done in future, as mentioned in the above sections, to expand and improve this model. One of the main difficulties of this study was linking data from the different matrices. The implementation of a more integrated data analysis approach to better link the effects in the different matrices, using techniques such as multi-block analyses, would greatly substantiate the results and aid in interpreting future work.

Results from this PhD and future work could furthermore contribute substantially to industry. Apart from a deeper understanding of grapevine biology, this information also has the potential to inform and rationalise design changes to management practices to manipulate berry and juice composition and thereby influence wine style. Moreover, the metabolite profiling in the various conditions also contributes to the identification of potential biomarkers directly linked to UVB light exposure that could become useful for screening purposes.

In conclusion, this PhD has contributed significantly to the current understanding of how UVB influences berry metabolites during development, highlighting mechanisms of acclimation and stress prevention. The approach of this study therefore generated new knowledge on berry metabolism in interaction with environmental stress factors. The results furthermore contributed to our understanding of how these microclimate variations impact juice characteristics and wine organoleptic properties and highlighted various novel and exciting responses. This thesis therefore contributed notably to the fields of viticulture and oenology and laid the groundwork for several potential future studies.

7.3 References

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